B6

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 August 2004 (26.08.2004)

PCT

(10) International Publication Number
WO 2004/072887 A2

(51) International Patent Classification[7]: G06F 19/00, C12Q 1/68

(21) International Application Number: PCT/GB2004/000538

(22) International Filing Date: 11 February 2004 (11.02.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/446,272 11 February 2003 (11.02.2003) US

(71) Applicant *(for all designated States except US)*: **SCIONA LIMITED** [GB/GB]; 79 George Street, Ryde, Isle Of Wight PO33 2JF (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROBERTS, Gareth** [GB/GB]; Sciona Limited, 79 George Street, Ryde, Isle Of Wight PO33 2JF (GB). **GILL-GARRISON, Rosalynn** [US/GB]; Sciona Limited, 79 George Street, Ryde, Isle Of Wight PO33 2JF (GB). **GRIMALDI, Keith** [GB/GB]; Sciona Limited, 79 George Street, Ryde, Isle Of Wight PO33 2JF (GB).

(74) Agents: **SUTCLIFFE, Nicholas** et al.; Mewburn Ellis, York House, 23 Kingsway, London, Greater London WC2 6HP (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPUTER-ASSISTED MEANS FOR ASSESSING LIFESTYLE RISK FACTORS OF AN INDIVIDUAL

(57) Abstract: The present invention relates to a computer assisted method of providing a personalized and confidential lifestyle advice plan for a human subject in which a sample from an individual is labelled with an identifier and analysed for the presence of alleles at one or more genetic loci which may be associated with lifestyle risk factors. The identifier is used to match the alleles identified at these loci with personal information relating to the lifestyle of the individual, which is stored in a secure location in a data processing means. Risk factors associated with the presence of each particular allele are generated by the data processing means and these risk factors are combined with the personal information relating to lifestyle in order to generate a personalized lifestyle advice plan. This may include for example recommended minimum and/or maximum amounts of food subtypes.

WO 2004/072887 A2

Computer-Assisted Means For Assessing Lifestyle Risk Factors of an Individual

Field of the Invention

The present invention relates to methods of assessing the susceptibility of an individual to a range of medical conditions. In particular, it relates to methods of assessing susceptibility to conditions that are associated with dietary and lifestyle risk factors and producing information and advice in a confidential manner.

Background to the Invention

For many decades, Governments, charities and health advisory bodies have issued health advice, for example relating to diet, exercise, smoking and sunbathing. This advice has been directed only at the public as a whole, or, at best, to groups such as the elderly, children and pregnant women. This advice can therefore only be very general and cannot, by its very nature, take account of the particular genotype of an individual. Moreover, in recent years, research findings on links between particular foods, drugs etc and medical conditions, have received large amounts of publicity, often causing health scares.

The factors that contribute to health status and susceptibility to medical conditions vary between populations and between individuals within populations, so it is often impossible for an individual to derive useful advice appropriate to his or her particular circumstances from such general reports and research. Variation in the alleles of certain genes, in particular those involved in dealing with environmental factors, leads to relatively greater or lesser susceptibility to particular medical

conditions for certain populations and individuals. Health advice appropriate for these populations and individuals, for example regarding diet and lifestyle, may be different to the advice that is appropriate to the public as a whole.

Summary of The Invention

In order to enable individuals to protect and manage their own health, there is a need for individuals to have personally- tailored information about risk factors which may be important to that individual's well-being and personally-tailored advice on reducing the risk of disease and ill health. Various aspects of the invention relate to improved methods for producing such personally tailored information and advice in a confidential manner.

Accordingly, the invention provides a computer-assisted method of providing a personalized lifestyle advice report for a human subject comprising:

(i) providing an identifier to a sample of DNA obtained from the human subject,

(ii) linking said identifier to personal details of the subject held in a secure location in a data processing means,

(iii) identifying individual alleles at one or more genetic loci in said sample to generate a first dataset of said human subject, wherein at least one allele of each genetic locus is known to be associated with factors which affect well-being or lifestyle in a positive or negative way,

(iv) linking said first dataset to said identifier,

(v) linking said first dataset and said personal details in said secure location by means of said identifier,

(vi) providing a second dataset on a data processing means, said second dataset comprising information correlating the presence of individual alleles at said genetic loci with a lifestyle risk factor;

(vii) providing a third dataset on a data processing means, said third dataset comprising information matching each said risk factor with at least one lifestyle recommendation;

(viii) determining the risk factors associated with said alleles of said human subject in said first dataset using said second dataset;

(ix) determining at least one appropriate lifestyle recommendation based on each identified risk factor from step (viii) using said third dataset,

(x) generating a personalized lifestyle advice plan based on said lifestyle recommendations and said personal details, and;

(xi) producing a report document containing said lifestyle advice plan.

Thus, the method allows individualised advice to be generated and disseminated in a confidential manner based on the unique genetic profile of an individual and the susceptibility to disease associated with the profile, taking into account personal information regarding the individual's lifestyle. By individually assessing the genetic make-up of the client, specific risk factors can be identified and dietary and other health advice tailored to the individual's needs. In a preferred embodiment, the lifestyle advice will include recommended minimum or maximum amounts of foodtypes. (Note that an amount may be 0).

Personal information, for example, concerning the sex and health of the individual and/or of the individual's family, may also provide indications that a particular polymorphism or group of polymorphisms associated with a particular condition should be investigated. Such information may therefore be used in selection of polymorphisms to be screened for in the method of the invention.

Personal information regarding the client may also be used in the determination of appropriate lifestyle recommendations in step (v) of the method. For example, recommendations relating to reducing susceptibility to prostate cancer are inappropriate when the subject is a women and recommendations relating to susceptibility to ovarian cancer are inappropriate when the subject is a men. Other personal details, such as information regarding the age, weight, alcohol consumption, smoking status, exercise levels and existing diet of the client may also be held in the data processing means and incorporated into the determination of appropriate lifestyle recommendations in step (v).

To ensure confidentiality, the first dataset and the subject's personal details are stored in a secure location within the data processing means, and are preferably encrypted. The separation of sample and personal details prior to genetic profiling, and subsequent linking of the first dataset with the personal details after profiling, using the identifier, allows potentially sensitive information to remain confidential.

The identifier may be any code or designation which uniquely identifies the sample and personal details. Preferably, the identifier is a barcode or serial number.

Preferably, the method provides for the confidential production of the report document containing the lifestyle advice plan (i.e. third party access to genetic and personal information is prevented). The report document may be produced, for example using standard printing techniques, and automatically sealed to maintain confidentiality until the human subject in question opens it.

For example, a method of the invention may comprise inserting and sealing said document in an enclosure in an automated process.

The enclosure may be an envelope or other wrapping and may be labelled with one or more of postal details of said human subject and said identifier. The enclosure containing the report document may then be sent to said human subject.

Preferably, the enclosure comprises an opening means or flap, which is non-resealable (i.e. the enclosure cannot be opened and then re-sealed). The enclosure may additional comprise a seal which is broken when the enclosure is opened.

The report document itself may comprise additional security measures. For example, sections of the report that contain personal information may be encased in a non-transparent wrapper, which must be broken or unsealed in order to

access these sections. Suitable wrappers include a sealed paper enclosure, which is openable by tearing or cutting along a perforated line of weakness, for example along one edge of the wrapper.

By lifestyle risk factors, it is meant risk factors associated with dietary factors, exposure to environmental factors, such as smoking, environmental chemicals or sunlight. Similarly lifestyle recommendations should be interpreted as relating to recommendations relating to dietary factors and exposure to environmental factors, such as smoking, environmental chemicals or sunlight. Disease susceptibility should be interpreted to include susceptibility to conditions such as allergies.

The method will involve assessing a variety of loci in order to give a broad view of susceptibility and possible means of minimising risk of medical conditions. For example, although individual polymorphisms may be considered biomarkers for individual cancer risk, the different biomarkers, when considered together, may also reveal a significant cancer risk.

The presence of a particular polymorphism may be indicative of increased susceptibilty to one disease while being indicative of decreased susceptibility to another disease. Therefore, it will be important to assess the risk factors associated with other polymorphisms to give meaningful advice on maintaining optimal health.

Preferred genes for which polymorphisms are identified include genes that encode enzymes responsible for detoxification of xenobiotics in Phase I metabolism; genes

that encode enzymes responsible for conjugation reactions in Phase II metabolism; genes that encode enzymes that help cells to combat oxidative stress; genes associated with micronutrient deficiency; genes that encode enzymes responsible for metabolism of alcohol; genes that encode enzymes involved in lipid and/or cholesterol metabolism; genes that encode enzymes involved in clotting; genes that encode enzymes related to susceptibility to metal toxicity; genes which encode proteins required for normal cellular metabolism and growth; genes that encode proteins involved with inflammation processes; and genes involved in calcium metabolism and bone growth and maintenance.

For example, genetic loci of genes encoding the following enzymes may be tested: 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), cystathione beta synthase (CBS), vitamin D receptor (VDR), collagen type I alpha (COL1A1), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), angiotensin converting enzyme (ACE), peroxisome proliferators activated receptor (PPAR-gamma 2), manganese superoxide dismutase (SOD2), extracellular superoxide dismutase (SOD3), glutathione S-transferase M1 (GSTM1), glutathione S-transferase theta1 (GSTT1), glutathione S-transferase pi (GSTP1), apolipoprotein C-III (APOC3), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), endothelial nitric oxide synthase (eNOS), factor 5 (F5) and apolipoprotein E (ApoE4).

The battery of genetic loci described herein are particularly useful in efficiently providing sufficient genetic information to allow lifestyle recommendations to

be made. Other aspects of the invention are directed at methods involving this battery of loci.

A computer-assisted method of providing a personalized lifestyle advice report for a human subject may comprise:
(i) providing a second dataset on a data processing means, said second dataset comprising information correlating the presence of individual alleles at genetic loci with a lifestyle risk factor, said genetic loci consisting of two or more of;

(a) genes that encode enzymes responsible for detoxification of xenobiotics in Phase I metabolism;

(b) genes that encode enzymes responsible for conjugation reactions in Phase II metabolism;

(c) genes that encode enzymes that help cells to combat oxidative stress;

(d) genes associated with micronutrient deficiency; and

(e) genes that encode enzymes responsible for metabolism of alcohol.

(f) genes that encode enzymes involved in lipid and/or cholesterol metabolism;

(g) genes that encode enzymes involved in clotting;

(h) genes that encode enzymes related to susceptibility to metal toxicity;

(i) genes which encode proteins required for normal cellular metabolism and growth; a

(j) genes that encode proteins involved with inflammation processes

(k) genes involved in calcium metabolism and bone growth and maintenance.

(ii) providing a third dataset on a data processing means, said second dataset comprising information matching each

said risk factor with at least one lifestyle recommendation;

(iii) inputting a first dataset identifying alleles at one or more of the genetic loci of said second dataset of said human subject;

(iv) determining the risk factors associated with said alleles of said human subject using said second dataset;

(v) determining at least one appropriate lifestyle recommendation based on each identified risk factor from step (iv) using said third dataset; and;

(vi) generating a personalized lifestyle advice plan based on said lifestyle recommendations.

The genetic loci tested may be loci of genes encoding the following: 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), cystathione beta synthase (CBS), vitamin D receptor (VDR), collagen type I alpha (COL1A1), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), angiotensin converting enzyme (ACE), peroxisome proliferators activated receptor (PPAR-gamma 2), manganese superoxide dismutase (SOD2), extracellular superoxide dismutase (SOD3), glutathione S-transferase M1 (GSTM1), glutathione S-transferase theta1 (GSTT1), glutathione S-transferase pi (GSTP1), apolipoprotein C-III (APOC3), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), endothelial nitric oxide synthase (eNOS), factor 5 (F5) and apolipoprotein E (ApoE4).

Methods of the invention may include the step of determining the presence of individual alleles at one or more genetic loci of the DNA in a DNA sample of the

subject, and constructing the dataset used in step (iii) using results of that determination.

Techniques for determining the presence or absence of individual alleles are known to the skilled person. They may include techniques such as hybridization with allele-specific oligonucleotides (ASO)(Wallace, 1981; Ikuta, 1987; Nickerson, 1990, Varlaan-de Vries, 1986, Saiki, 1989 and Zhang, 1991) allele specific PCR (Newton 1989, Gibbs, 1989), solid-phase minisequencing (Syvanen, 1993), oligonucleotide ligation assay (OLA) (Wu, 1989, Barany, 1991; Abravaya, 1995), 5' fluorogenic nuclease assay (Holland, 1991 & 1992, Lee, 1998) US patents 4,683,202, 4,683,195, 5,723,591 and 5,801,155, or Restriction fragment length polymorphism (RFLP) (Donis-Keller, 1987).

In a preferred embodiment, the genetic loci are assessed via a specialised type of PCR used to detect polymorphisms, commonly referred to as the Taqman® assay, in which hybridisation of a probe comprising a fluorescent reporter molecule, a fluorescent quencher molecule and a minor groove binding chemical to a region of interest is detected by removal of quenching of the fluorescent molecule and detection of resultant fluorescence. Details are given below.

In another embodiment, the genetic loci are assessed via hybridisation with allele-specific oligonucleotides, the allele specific oligonucleotides being preferably arranged as an array of oligonucleotide spots stably associated with the surface of a solid support.

The arrays suitable for use in the method of the invention form a further aspect of the present invention.

In order to assay the sample for the alleles to be identified the fragments of DNA comprising the gene(s) of interest may be amplified to produce a sufficient amount of material to be tested.

The present inventors have designed a number of specific primer sets for amplification of gene regions of interest. Such primers may be used in pairs to isolate a particular region of interest in isolation. Therefore in a further aspect of the invention, there is provided a primer having a sequence selected from SEQ ID NOS: 1 to 54. In another aspect, there is provided a primer pair comprising primers having SEQ ID NO:n, where n is an odd number from 1 to 53 in conjunction with a primer having SEQ ID NO:(n+1).

Preferably, however, the primer sets will be used together with other primer sets to provide multiplexed amplification of a number of regions to allow determination of a number of polymorphisms from the same sample. Therefore in a further aspect of the invention, there is provided a primer set comprising at least 5, more preferably 10, 15 primer pairs selected from SEQ ID NOS: 1 to 54.

Brief Description of the Tables

Table 1 shows the primers used to amplify genetic loci in methods of certain embodiments of the invention.

Table 2 shows examples of probes for identifying alleles at genes of interest.

Table 3 shows examples of databases 1 and 2 that may be used in an embodiment of the present invention.

Table 4 is a flow chart illustrating an embodiment of the invention.

Table 5 shows an example of a report document containing personalised advice from a lifestyle advice plan.

Table 6 shows an example of a genetic profile as summarised in a report document.

Table 7 shows an example of a report document containing a summary of personalised lifestyle advice that employs pictoral icons to facilitate comprehension of the information by the individual.

Detailed Description of the Invention

Selection of Genetic Polymorphisms for Datasets

The correct selection of genetic polymorphisms is important to the provision of accurate and meaningful advice without excessive testing. Markers for polymorphisms of one or more of the following classes of genes may be used in methods of the invention:

The first dataset of methods of the invention may comprise information relating to two or more alleles of two, three, four or more, more preferably all, of the genetic loci of the following genes:

(a) genes that encode enzymes responsible for detoxification of xenobiotics in Phase I metabolism;

(b) genes that encode enzymes responsible for conjugation reactions in Phase II metabolism;

(c) genes that encode enzymes that help cells to combat oxidative stress;

(d) genes associated with micronutrient deficiency;

(e) genes that encode enzymes responsible for metabolism of alcohol;

(f) genes that encode enzymes involved in lipid and/or cholesterol metabolism;

(g) genes that encode enzymes involved in clotting;

(h) genes that encode enzymes related to susceptibility to metal toxicity;

(i) genes which encode proteins required for normal cellular metabolism and growth;

(j) genes that encode proteins involved with inflammation processes; and,

(k) genes involved in calcium metabolism and bone growth and maintenance.

The dataset will preferably comprise information relating to two or more alleles of at least two, more preferably all of the genetic loci of genes selected from the group comprising categories a - k as described above, for example, a+b, a+c, a+d, a+e, a+f, a+g, a+h, a+i, a+j, a+k, b+c, b+d, b+e etc., c+d, c+e etc, d+e, d+f etc, e+f, e+g etc, f+g, f+h etc., g+h, g+i, g+k, h+i, h+k. Where the dataset comprises information relating to two or more alleles of at least two genetic loci, it is preferred that at least one of the genetic loci is of category d, due to the central role of micronutrients in the maintenance of proper cellular growth and DNA repair, and due to the association of micronutrient metabolism or utilisation disorders with several different types of diseases (Ames 1999; Perera, 2000; Potter, 2000). More preferably, the dataset will preferably comprise information relating to

two or more alleles of at least three genetic loci selected from the group comprising categories a - k as described above. Where the dataset comprises information relating to alleles of at least three genetic loci, it is preferred that at least two of the genetic loci are of categories d and e. Information relating to polymorphisms present in both of these categories is particularly useful due to the effects of alcohol consumption and metabolism on the efficiency of enzymes related to micronutrient metabolism and utilisation (Ulrich, 1999). In a further preferred embodiment, where the dataset comprises information relating to alleles of at least three genetic loci, it is preferred that at least two of the genetic loci are of categories a and b due to the close interaction of Phase I and Phase II enzymes in the metabolism of xenobiotics. Even more preferably, the dataset will comprise information relating to two or more alleles of at least four genetic loci of genes selected from the group comprising categories a - k as defined above, for example, a+b+c+d, a+b+c+e, a+b+d+e, a+c+d+e, b+c+d+e etc. Where the dataset comprises information relating to alleles of at least four genetic loci, it is preferred that at least three of the genetic loci are of categories d and e and f. Information relating to polymorphisms present in these three categories is particularly useful due to the strong correlation of polymorphisms of these alleles with coronary artery disease due to the combined effects of altered micronutrient utilisation, affected adversely by alcohol metabolism, together with imbalances in fat and cholesterol metabolism. Further, where the dataset comprises information relating to alleles of at least five genetic loci, it is preferred that at least four of the genetic loci are of categories a, b, d and e. Information relating to polymorphisms present

in these four categories is particularly useful due to the combined effects of micronutrients utilisation, alcohol metabolism, Phase 1 metabolism of xenobiotics and Phase II metabolism on the further metabolism and excretion of potentially harmful metabolites produced in the body (Taningher, 1999; Ulrich, 1999). Similarly, the dataset may comprise information relating to two or more alleles of at least five, for example a, b, d, e and f, six, seven, eight, nine or ten genetic loci of genes selected from the group comprising categories a - k as defined above.

Preferably, the dataset will comprise information relating to two or more alleles of one or more genetic loci of genes selected from each member of the group comprising categories a - k as described above. More preferably, the first dataset comprises information relating to two or more alleles of the genetic loci of genes encoding each of the 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), cystathione beta synthase (CBS), vitamin D receptor (VDR), collagen type I alpha (COL1A1), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), angiotensin converting enzyme (ACE), peroxisome proliferators activated receptor (PPAR-gamma 2), manganese superoxide dismutase (SOD2), extracellular superoxide dismutase (SOD3), glutathione S-transferase M1 (GSTM1), glutathione S-transferase theta1 (GSTT1), glutathione S-transferase pi (GSTP1), apolipoprotein C-III (APOC3), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), endothelial nitric oxide synthase (eNOS), factor 5 (F5) and apolipoprotein E (ApoE4).

Detoxification of xenobiotics occurs in two phases in humans:

Phase I metabolism involves the addition of an oxygen atom or a nitrogen atom to lipophilic (fat soluble) compounds, such as steroids, fatty acids, xenobiotics (from external sources like diet, smoke, etc.) so that they can be conjugated by the Phase II enzymes (thus made water-soluble) and excreted from the body (Hirvonen, 1999). Individuals with genetic polymorphisms correlated with cancer risk in these genes should avoid consumption of char-grilled foods, smoked fish, well-done red meat whether grilled or pan-fried (Sinha, 1999). They should also increase consumption of food products known to increase Phase II metabolism so the products of Phase I metabolism may be cleared more efficiently.

Glutathione-S-transferases (GSTM1, GSTP1, GSTT1) catalyse the reaction of electrophilic compounds with glutathione so the compounds may be excreted from the body. The enzymes belong to a super-family with broad and overlapping substrate specificities. Glutathione-S-transferases provide a major pathway of protection against chemical toxins and carcinogens and are thought to have evolved as an adaptive response to environmental insult, thus accounting for their wide substrate specificity (Hirvonen, 1999). There are 4 family members: alpha, mu, theta, and pi, also designated as A, M, T and P. Polymorphisms have been identified in each family (Perera, 2000). Individuals with low glutathione-S-transferase activity should avoid meats cooked at higher temperatures as above, and increase fruit and vegetable consumption. Cruciferous vegetables such as broccoli and members of the allium family such as

garlic and onion have been shown to be potent inducers of these enzymes, which would be expected to increase clearance of toxic substances from the body (Cotton, 2000; Giovannucci, 1999).

GSTmu, has 3 alleles: null, a, which is considered to be the wild type, and b, which comprises a C534G substitution, with no functional difference between the a and b alleles. The GSTmu sub-type has the highest activity of the 4 types and is predominately located in the liver (Hirvonen, 1999). Approximately half of the population has a complete deletion of this gene with a corresponding risk of lung, bladder, breast, liver, and oral cavity cancer (Shields, 2000; Perera, 2000). It has been estimated that 17% of all lung and bladder cancers may be attributable to GSTM1 null genotypes (Hirvonen, 1999). GSTM1 null genotype together with a highly active CYP1A1 polymorphism has been linked to a very high cancer risk in several studies (Rojas, 2000; Shields, 2000). The GSTM1 gene is located on chromosome 1p13.3. (Cotton, 2000).

GSTpi gene is located on chromosome 11q13. This sub-type is known to metabolise many carcinogenic compounds and is the most abundant sub-type in the lungs (Hirvonen, 1999). Two single nucleotide polymorphisms have been linked to cancer to date GSTP1*B, which comprises an A313G substitution, and GSTP1*C, which comprises a C341T substitution. The enzymes of these polymorphic genes have decreased activity compared to the wild type and a corresponding increased risk of bladder, testicular, larynx and lung cancer (Harries, 1997; Matthias, 1998; Ryberg, 1997).

GSTtheta gene is on chromosome 22q11.2 and is deleted in approximately 20% of the Caucasian population. The enzyme is found in a variety of tissues, including red blood cells, liver, and lung (Potter, 1999). The deletion is associated with an increased risk of lung, larynx and bladder cancers (Hirvonen, 1999). Links with GSTM1 null genotypes are currently being searched, as it is believed that individuals that have both GSTM1 and GSTT1 alleles deleted will have a greatly increased risk of developing cancer (Potter, 1999).

Specific examples of genes that encode enzymes that help cells to combat oxidative stress include genes encoding manganese superoxide dismutase (MnSOD or SOD2 gene) and extracellular superoxide dismutase (SOD3). Manganese superoxide dismutase is an enzyme that destroys free radicals or a free-radical scavenger. The gene is located on chromosome 6q25.3, but the enzyme is found within the mitochondria of cells. There are 2 polymorphisms linked to cancer to date, an Ile 58Thr allele, which comprises an T175C substitution, and a Val(-9)Ala allele, which comprises a T(-28)C substitution,. A study of premenopausal women found a four-fold increased risk of breast cancer in individuals with the Val(-9)Ala polymorphism and the highest risk within this group is found in women who consumed low amounts of fruits and vegetables (Ambrosone, 1999). This polymorphism occurs in the signal sequence of the amino acid chain. The signal sequence ensures transport of the enzyme into the mitochondria of the cell, and so the polymorphism is believed to reduce the amount of enzyme delivered to the mitochondria (Ambrosone, 1999). The mitochondria is commonly referred to as the workhorse of the cell, where the energy-yielding reactions take

place. This is the site of many oxidative reactions, so many free radicals are generated here. Individuals with low activity of this enzyme should be advised to take antioxidant supplements and increase consumption of fruits and vegetables (Giovannucci, 1999; Perera, 2000).

Genes associated with micronutrient deficiency include the gene encoding 5,10-methylenetetrahydrofolatereductase (MTHFR) activity. 5,10-methylenetetrahydrofolate reductase is active in the folate-dependent methylation of DNA precursors. Low activity of this enzyme leads to an increase of uracil incorporation into DNA (instead of thymine) (Ames, 1999). The MTHFR gene is polymorphic and has been linked to colon cancer, adult acute lymphocytic leukaemia and infant leukaemia (Ames, 1999; Perera, 2000; Potter, 2000). Both the wt and polymorphic alleles have been linked to disease, each being dependent on levels of folate in the diet. Approximately 35% of the Caucasian population has genetic polymorphisms at this locus with corresponding risk of colon cancer (Shields, 2000). Polymorphisms at this locus include those with a C677T or A1298C substitution. Dietary recommendations for individuals lacking in MTHFR activity include taking supplements with folate and increasing consumption of fruit and vegetables (Ames, 1999). Low levels of vitamins B12 and B6 have been associated with low MTHFR activity and increased cancer risk, so individuals should increase intake of these vitamins; B12 is found primarily in meat and B6 is found in whole grains, cereals, bananas, and liver (Ames, 1999). Alcohol has a deleterious effect on folate metabolism, affecting individuals with the A1298C polymorphism most severely (Ulrich, 1999). These individuals should be advised to avoid alcohol.

Genes that encode enzymes involved in lipid and/or cholesterol metabolism include genes encoding cholesteryl ester transfer protein e.g. the CETP gene, polymorphisms of which genes are associated with altered susceptibility to coronary artery disease (CAD)((Raknew, 2000; Ordovas, 2000); apolipoprotein E(ApoE), polymorphisms of which genes are associated with altered susceptibility to CAD and Alzheimer's disease (Corbo,1999; Bullido, 2000); or apolipoprotein C, III (ApoC-III), polymorphisms of which genes are associated with altered susceptibility to CAD, hypertension and insulin resistance (Salas, 1998).

Genes that encode enzymes involved in clotting include genes encoding angiotensin converting enzyme (ACE), polymorphisms of which genes are associated with altered susceptibility to hypertension (Brand 2000;de Padua Mansur, 2000), and factor V.

Genes that encode enzymes related to susceptibility to metal toxicity include genes encoding the vitamin D receptor, polymorphisms of which genes are associated with altered susceptibility to osteoporosis, tuberculosis, Graves disease, COPD, and early periodontal disease (Ban, 2000; Wilkinson, 2000; Gelder, 2000; Miki, 1999; Hennig, 1999); cystathionine-beta-synthase, polymorphisms of which genes are associated with altered susceptibility to CAD (Tsai, 1999); methionine synthase (B12 MS) and methionine synthase reductase, polymorphisms of which genes are associated with altered susceptibility to CAD (Tsai, 1999); and transforming growth factor alpha (TGFα), polymorphisms of which genes are associated with altered susceptibility to CAD and cancers (Yokota, 2000).

Genes involved in calcium metabolism and bone growth and maintenance include IL-6, polymorphisms of which genes are associated with altered susceptibility to osteoporosis.

Detection of Polymorphisms

As described above, the method of the invention may include the step of analysing a DNA sample of a human subject in order to construct the dataset to be used in the method of the invention.

Testing of Samples

Collection of Tissue Samples

DNA for analysis using the method or arrays of the invention can be isolated from any suitable client or patient cell sample. For convenience, it is preferred that the DNA is isolated from cheek (buccal) cells. This enables easy and painless collection of cells by the client, with the convenience of being able to post the sample to the provider of the genetic test without the problems associated with posting a liquid sample.

Cells may be isolated from the inside of the mouth using a disposable scraping device with a plastic or paper matrix "brush", for example, the C.E.P. Swab™ (Life Technologies Ltd., UK). Cells are deposited onto the matrix upon gentle abrasion of the inner cheek, resulting in the collection of approximately 2000 cells (Aron, 1994). The paper brush can then be left to dry completely, ejected from the handle placed into a microcentrifuge tube and posted by the client or patient to the provider of the genetic test.

Isolation of DNA from Samples

DNA from the cell samples can be isolated using conventional procedures. For example DNA may be immobilised onto filters, column matrices, or magnetic beads. Numerous commercial kits, such as the Qiagen QIAamp kit (Quiagen, Crawley, UK) may be used. Briefly, the cell sample may be placed in a microcentrifuge tube and combined with Proteinase K, mixed, and allowed to incubate to lyse the cells. Ethanol is then added and the lysate is transferred to a QIAamp spin column from which DNA is eluted after several washings.

The amount of DNA isolated by the particular method used may be quantified to ensure that sufficient DNA is available for the assay and to determine the dilution required to achieve the desired concentration of DNA for PCR amplification. For example, the desired target DNA concentration may be in the range 10 ng and 50 ng. DNA concentrations outside this range may impact the PCR amplification of the individual alleles and thus impact the sensitivity and selectivity of the polymorphism determination step.

The quantity of DNA obtained from a sample may be determined using any suitable technique. Such techniques are well known to persons skilled in the art and include UV (Maniatis, 1982) or fluorescence based methods. As UV methods may suffer from the interfering absorbance caused by contaminating molecules such as nucleotides, RNA, EDTA and phenol and the dynamic range and sensitivity of this technique is not as great as that of fluorescent methods, fluorescence methods are preferred. Commercially available

fluorescence based kits such as the PicoGreen dsDNA Quantification (Molecular Probes, Eugene, Oregon, USA).

Primers

Prior to the testing of a sample, the nucleic acids in the sample may be selectively amplified, for example using Polymerase Chain Reaction (PCR) amplification as described in U.S. patent numbers 4,683,202 and 4,683,195.

Preferred primers for use in the present invention are from 18 to 23 nucleotides in length, without internal homology or primer-primer homology.

Furthermore, to ensure amplification of the region of interest and specificity, the two primers of a pair are preferably selected to hybridise to either side of the region of interest so that about 150 bases in length are amplified, although amplification of shorter and longer fragments may also be used. Ideally, the site of polymorphism should be at or near the centre of the region amplified.

Table 1 provides preferred examples of primer pairs which may be used in the invention, particularly when the Taqman® assay is used in the method of the invention. The primers are shown together with the gene targets. Wild-type (wt) probes and polymorphism probes suitable for use in the Taqman® assay for each gene target may be designed using conventional techniques in accordance with the instructions from the Taqman® assay suppliers.

The primers and primer pairs form a further aspect of the invention. Therefore the invention provides a primer having

a sequence selected from SEQ ID NOS: 1 to 54. In another aspect, there is provided a primer pair comprising primers having SEQ ID NO: n, where n is an odd number from 1 to 54 in conjunction with a primer having SEQ ID NO:(n+1).

In a preferred embodiment of the invention, multiplexed amplification of a number of sequences are envisioned in order to allow determination of the presence of a plurality of polymorphisms using, for example the DNA array method. Therefore, primer pairs to be used in the same reaction are preferably selected by position, similarity of melting temperature, internal stability, absence of internal homology or homology to each other to prevent self-hybridisation or hybridisation with other primers and lack of propensity of each primer to form a stable hairpin loop structure. Thus, the sets of primer pairs to be coamplified together preferably have approximately the same thermal profile, so that they can be effectively coamplified together. This may be achieved by having groups of primer pairs with approximately the same length and the same G/C content.

Therefore in a further aspect of the invention, there is provided a primer set comprising at least 5, more preferably 10, 15 primer pairs selected from SEQ ID NO: 1 to 54.

Having obtained a sample of DNA, preferably with amplified regions of interest, individual polymorphisms may be identified. Identification of the markers for the polymorphisms involves the discriminative detection of allelic forms of the same gene that differ by nucleotide substitution, or in the case of some genes, for example the

GSTM1 and GSTT1 genes, deletion of the entire gene. Methods for the detection of known nucleotide differences are well known to the skilled person. These may include, but are not limited to:

- Hybridization with allele-specific oligonucleotides (ASO), (Wallace, 1981; Ikuta, 1987; Nickerson, 1990, Varlaan, 1986, Saiki, 1989 and Zhang, 1991).
- Allele specific PCR, (Newton 1989, Gibbs, 1989).
- Solid-phase minisequencing (Syvanen, 1993).
- Oligonucleotide ligation assay (OLA) (Wu, 1989, Barany, 1991; Abravaya, 1995).
- The 5′ fluorogenic nuclease assay (Holland, 1991 & 1992, Lee, 1998, US patents 4,683,202, 4,683,195, 5,723,591 and 5,801,155).
- Restriction fragment length polymorphism (RFLP), (Donis-Keller, 1987).

In a preferred embodiment, the genetic loci are assessed via a specialised type of PCR used to detect polymorphisms, commonly referred to as the Taqman® assay and performed using an AB7700 instrument (Applied Biosystems, Warrington, UK). In this method, a probe is synthesised which hybridises to a region of interest containing the polymorphism. The probe contains three modifications: a fluorescent reporter molecule, a fluorescent quencher molecule and a minor groove-binding chemical to enhance binding to the genomic DNA strand. The probe may be bound to either strand of DNA. For example, in the case of binding to the coding strand, when the Taq polymerase enzyme begins to synthesise DNA from the 5′ upstream primer, the polymerase will encounter the probe and begin to remove bases from the probe one at a time using a 5′-3′

exonuclease activity. When the base bound to the fluorescent reporter molecule is removed, the quencher molecule no longer quenches the fluorescent molecule and the molecule will begin to fluoresce. This type of reaction can only take place if the probe has hybridised perfectly to the matched genomic sequence. As successive cycles of amplification take place, i.e. more probes and primers are bound to the DNA present in the reaction mixture, the amount of fluorescence will increase and a positive result will be detected. If the genomic DNA does not have a sequence that matches the probe perfectly, no fluorescent signal is detected.

Arrays

In a preferred embodiment of the invention, hybridisation with allele specific oligonucleotides is conveniently carried out using oligonucleotide arrays, preferably microarrays, to determine the presence of particular polymorphisms.

Such microarrays allow miniaturisation of assays, e.g. making use of binding agents (such as nucleic acid sequences) immobilised in small, discrete locations (microspots) and/or as arrays on solid supports or on diagnostic chips. These approaches can be particularly valuable as they can provide great sensitivity (particularly through the use of fluorescent labelled reagents), require only very small amounts of biological sample from individuals being tested and allow a variety of separate assays to be carried out simultaneously. This latter advantage can be useful as it provides an assay for different a number of polymorphisms of one or more genes to be carried out using a single sample. Examples of

techniques enabling this miniaturised technology are provided in WO84/01031, WO88/1058, WO89/01157, WO93/8472, WO95/18376/ WO95/18377, WO95/24649 and EP-A-0373203, the subject matter of which are herein incorporated by reference.

DNA microarrays have been shown to provide appropriate discrimination for polymorphism detection. Yershov, 1996; Cheung, 1999 and Schena 1999 have described the principles of the technique. In brief, the DNA microarray may be generated using oligonucleotides that have been selected to hybridise with the specific target polymorphism. These oligonucleotides may be applied by a robot onto a predetermined location of a glass slide, e.g. at predetermined X,Y cartesian coordinates, and immobilised. The PCR product (e.g. fluorescently labelled RNA or DNA) is introduced on to the DNA microarray and a hybridisation reaction conducted so that sample RNA or DNA binds to complementary sequences of oligonucleotides in a sequence-specific manner, and allow unbound material to be washed away. Gene target polymorphisms can thus be detected by their ability to bind to complementary oligonucleotides on the array and produce a signal. The absence of a fluorescent signal for a specific oligonucleotide probe indicates that the client does not have the corresponding polymorphism. Of course, the method is not limited to the use of fluorescence labelling but may use other suitable labels known in the art. the fluorescence at each coordinate can be read using a suitable automated detector in order to correlate each fluorescence signal with a particular oligonucleotide.

Oligonucleotides for use in the array may be selected to span the site of the polymorphism, each oligonucleotide comprising one of the following at a central location within the sequence:

- wild-type or normal base at the position of interest in the leading strand
- wild-type or normal base at the position of interest in the lag (non-coding) strand
- altered base at the position of interest in the leading strand
- altered complementary base at the position of interest in the lag strand

The arrays used in the present method form another independent aspect of the present invention. Arrays of the invention comprise a set of two or more oligonucleotides, each oligonucleotide being specific to a sequence comprising one or more polymorphisms of a gene selected from the group comprising categories a-k as defined above.

Preferably, the array will comprise oligonucleotides each being specific to a sequence comprising one or more polymorphisms of an individual gene of at least two different categories a-k as defined above, for example a+b (i.e. at least one oligonucleotide specific for a sequence comprising one or more polymorphisms of a first gene, the first gene being of category a and at least one oligonucleotide specific for a sequence comprising one or more polymorphisms of a second gene, the second gene being of category b), a+c, a+d, a+e, a+f, a+g, a+h, a+i, a+j, a+k, b+c, b+d, b+e etc., c+d, c+e etc, d+e, d+f etc, e+f, e+g etc, f+g, f+h etc., g+h, g+i, g+k, h+i, h+k. Where the

array comprises two or more oligonucleotides, it is preferred that at least one of the oligonucleotides is an oligonucleotide specific for a sequence of a polymorphism of a gene of category d, due to the central role of micronutrients in the maintenance of proper cellular growth and DNA repair, and due to the association of micronutrient metabolism or utilisation disorders with several different types of diseases (Ames 1999; Perera, 2000; Potter, 2000). More preferably, the array will comprise oligonucleotides each being specific to a sequence comprising one or more polymorphisms of an individual gene of at least three different categories a-k as defined above, for example, a+b+c, a+b+d, a+b+e, a+b+f, a+b+g, a+b+h, a+b+i, a+b+j, a+b+k a+c+d, a+c+e etc, a+d+e, etc, b+c+d, etc, c+d+e etc, d+e+f etc, and all other combinations of three categories. Where the array comprises three or more oligonucleotides, it is preferred that at least two of the oligonucleotides are oligonucleotides specific for a sequence of a polymorphism of a gene of categories d and e. Information relating to polymorphisms present in both of these categories is particularly useful due to the effects of alcohol consumption and metabolism on the efficiency of enzymes related to micronutrient metabolism and utilisation (Ulrich, 1999). In a further preferred embodiment where the array comprises three or more oligonucleotides, it is preferred that at least two of the oligonucleotides are oligonucleotides specific for a sequence of a polymorphism of a gene of c categories a and b due to the close interaction of Phase I and Phase II enzymes in the metabolism of xenobiotics. Even more preferably, the array will comprise oligonucleotides each being specific to a sequence comprising one or more polymorphisms of an individual gene of at least four different categories a-k

as defined above, for example, a+b+c+d, a+b+c+e, a+b+d+e, a+c+d+e, b+c+d+e etc. Where the array comprises four or more oligonucleotides, it is preferred that at least three of the oligonucleotides are oligonucleotides specific for a sequence of a polymorphism of a gene of categories d and e and f. Information relating to polymorphisms present in these three categories is particularly useful due to the strong correlation of polymorphisms of these alleles with coronary artery disease due to the combined effects of altered micronutrient utilisation, affected adversely by alcohol metabolism, together with imbalances in fat and cholesterol metabolism. Where the array comprises five or more oligonucleotides, it is preferred that at least four of the oligonucleotides are oligonucleotides specific for a sequence of a polymorphism of a gene of categories a, b, d and e. Information relating to polymorphisms present in these four categories is particularly useful due to the combined effects of micronutrients utilisation, alcohol metabolism, Phase 1 metabolism of xenobiotics and Phase II metabolism on the further metabolism and excretion of potentially harmful metabolites produced in the body (Taningher, 1999; Ulrich, 1999). Similarly, the array may comprise oligonucleotides each being specific to a sequence comprising one or more polymorphisms of an individual gene of at least five, for example a, b, d, e and f, six, seven, eight, nine or ten different categories a-k as defined above.

Most preferably, the array will comprise oligonucleotides each being specific to a sequence comprising one or more polymorphisms of an individual gene of each of categories a-k as defined above.

In one preferred embodiment, the array comprises oligonucleotides each being specific to a sequence comprising one or more polymorphisms of individual genes, the individual genes comprising each member of the group of genes encoding the enzymes: 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), cystathione beta synthase (CBS), vitamin D receptor (VDR), collagen type I alpha (COL1A1), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), angiotensin converting enzyme (ACE), peroxisome proliferators activated receptor (PPAR-gamma 2), manganese superoxide dismutase (SOD2), extracellular superoxide dismutase (SOD3), glutathione S-transferase M1 (GSTM1), glutathione S-transferase thetal (GSTT1), glutathione S-transferase pi (GSTP1), apolipoprotein C-III (APOC3), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), endothelial nitric oxide synthase (eNOS), factor 5 (F5) and apolipoprotein E (ApoE4).

Examples of oligonucleotides suitable for use in an array are shown in table 2.

Advice decision tree

The results of genetic polymorphism analysis may be used to correlate the genetic profile of the donor of the sample with disease susceptibility using the first dataset, which provides details of the relative disease susceptibility associated with particular polymorphisms and their interactions. The risk factors identified using dataset 1 can then be matched with dietary and other lifestyle recommendations from dataset 2 to produce a lifestyle advice plan individualised to the genetic profile of the

donor of the sample. Examples of datasets 1 and 2 which may be used to generate such advice is shown in Table 3.

To enable appropriate advice to be tailored to particular susceptibilities, a ranking system is preferably used to provide an indication of the degree of susceptibility of a specific polymorph to risk of cancer(s) and/or other conditions. The ranking system may be designed to take into account of homozygous or heterozygous alleles in the client's sample, i.e. the same or different alleles being present in diploid nucleus. Five categories which may be used are summarised below:

(i) Reduced susceptibility: where an allele has been shown to reduce susceptibility.

(ii) Normal susceptibility: where allele has been shown to have a normal susceptibility of risk to cancer(s) or disease. This is generally the homozygous wild type allele or a polymorphism that has been shown to have similar function.

(iii) Moderate susceptibility: where a heterozygous genotype is present that contains the wild type of the allele (i.e. normal susceptibility) and an allele of the polymorphism known to give rise to higher susceptibility to specific cancer(s) or disease.

(iv) High susceptibility: where a homozygous genotype that contains the polymorphism is present with a higher risk of cancer susceptibility.

(v) Higher susceptibility: where a higher susceptibility has been observed for specific cancer(s) or disease due to the combined effects of two or more different gene targets.

Using dataset 1, a susceptibility may be assigned to each polymorphism identified and, from dataset 2, a lifestyle recommendation corresponding to each susceptibility identified may be assigned. For example, if an individual is found to have the polymorphism MTHFR C677T, the decision tree may indicate that the there is an enhanced susceptibility of certain forms of cancer. Recommendations appropriate to minimising the risk of these cancers are then generated. For example, the recommendations may be to avoid particular foods associated with increased risk and to increase consumption of other foods associated with a protective effect against such cancers.

The totality of recommendations is combined to generate a lifestyle advice plan individualised to the donor of the sample. The decision tree is preferably arranged to recognise particular combinations of polymorphisms and/or susceptibilities which interact either positively to produce a susceptibility greater than would be expected from the risk factors associated with each individually, and/or, which interact negatively to reduce the susceptibility associated with each individually. Where such combinations are identified, the advice generated can be tailored accordingly.

In generating the advice, other factors such as information concerning the sex and health of the individual and /or of the individual's family, age, weight, exercise levels, smoking status, alcohol consumption, and existing diet may be used in the determination of appropriate lifestyle recommendations.

The report may comprise a lifestyle summary which contains the key advice (see Table 5) and a summary of the genetic variations found (Table 6). These sections may be arranged in an easy to read format. Visual icons which symbolize the results may be used to enhance the clarity and ease of understanding of the summary (Table 7). These icons are especially useful when dealing with a multi-cultural or international client base, as the symbols are readily understood and do not require a working knowledge of the English language.

Aspects of the present invention will now be illustrated with reference to the accompanying tables described above and the experimental exemplification below, by way of example and not limitation. Further aspects and embodiments will be apparent to those of ordinary skill in the art. All documents mentioned in this specification are hereby incorporated herein by reference.

Experimental

Example 1 Preparation of DNA Sample

DNA is prepared from a buccal cell sample on a brush using a Qiagen QIAamp kit according to the manufacturer's instructions (Qiagen, Crawley, UK). Briefly, the brush is cut in half and one half stored at room temperature in a sealed tube in case retesting is required. The other half of the brush is placed in a microcentrifuge tube.

The sample is assigned a barcode. Personal details of the supplier of the sample are stored in a computer and referenced also with the barcode, to allow the details to be linked with the subjects genetic profile after analysis.

400µl PBS is added and the brush allowed to rehydrate for 45 minutes at room temperature. Quiagen lysis buffer and Proteinase K is then added, the contents are mixed, and allowed to incubate at 56 C for 15 minutes to lyse the cells. Ethanol is added and the lysate transferred to a QIAamp spin column from which DNA is eluted after several washings.

Example 2 Quantification of DNA

In order to check that sufficient DNA has been isolated, a quantification step is carried out using the PicoGreen dsDNA Quantification kit (Molecular Probes, Eugene, Oregon, USA).

Briefly, client DNA samples are prepared by transferring a 10 µl aliquot into a microcentrifuge tube with 90µl TE. 100 µl of the working PicoGreen dsDNA quantification reagent is added, mixed well, and transferred into a black 96 well plate with flat well bottoms. The plate is then incubated for 5 minutes in the dark before a fluorescent reading is taken. The quantity of DNA present in the clients' samples is determined by extrapolating from a calibration plot prepared using DNA standards.

A quantity of DNA in the range of 5-50ng total is used in the subsequent PCR step. Remaining client DNA sample is stored at -20°C for retesting if required.

Example 3 Taqman® Assay to Identify the MTHFR A1298C polymorphism

The modified reaction mixture contains Taq polymerase (1.25 units/µl), optimised PCR buffer, dNTP (200µM each), 2mM

$MgCl_2$ and primer pairs SEQ ID NOS: 3 and 4 and polymorphism probe agtgaagcaagtgtc (SEQ ID NO: 55).

The reaction mixture is initially incubated for 10 minutes at 50°C, then 5 minutes at 95°C, followed by 40 cycles of 1 minute of annealing at between 55°C and 60°C and 30 seconds of denaturation at 95°C. Both during the cycles and at the end of the run, fluorescence of the released reporter molecules of the probe is measured by an integral CCD detection system of the AB7700 thermocycler. The presence of a fluorescent signal that increases in magnitude through the course of the run indicates a positive result.

The assay is then repeated with the same primer pair and wt probe cagtgaagaaagtgtc (SEQ ID NO: 56). If the sample is homozygous for the polymorphism, no fluorescence signal is seen with the wt probe. However, if the sample is heterozygous for the polymorphism, a fluorescence signal is also seen with the wt probe. If single reporter results from homozygous wt, homozygous polymorphic and heterozygous polymorphic samples are plotted are plotted on an X/Y axis, the homozygous alleles will cluster at opposite ends of the axes relative to each reporter, and the heterozygous alleles will cluster at a midway region.

Example 4 DNA Array method for identifying polymorphisms for Identifying multiple polymorphisms

a) PCR amplification

The PCR reaction mix contains Taq polymerase (1.25 units/reaction), optimised PCR buffer, dNTP's (200μM each) and $MgCl_2$ at an appropriate concentration of between 1 and 4 mM, and 40 pmol of each primer (SEQ ID NOS: 1 to 54) for amplification of control and sample DNA.

The reaction mixture is initially incubated at 95°C for 1 minute, and then subjected to 45 cycles of PCR in a MWG TC9600 thermocycler (MWG-Biotech-AG Ltd., Milton Keynes, UK) as follows:
annealing 50°C, 1 minute
polymerisation 73°C., 1 minute
denaturation 95°C., 30 seconds.
After a further annealing step at 50°C, 1 minute, there is a final polymerisation step at 73°C for 7 minutes.

(Instead of the MWG TC9600 thermocycler, other thermocyclers, such as the Applied Biosystems 9700 thermocycler (Applied Biosystems, Warrington, UK), may be used.

After amplification of the target genes, generation of product is checked by electrophoresis separation using 2% agarose gel, or a 3.5% NuSieve agarose gel.

The PCR amplification products are then purified using the Qiagen QIAquick PCR Purification Kit (Qiagen, Crawley, UK) to remove dNTPs, primers, and enzyme from the PCR product. The PCR product is layered onto a QIAquick spin column, a vacuum applied to separate the PCR product from the other reaction products and the DNA eluted in buffer.

b) RNA transcription and Fluorescent Labelling of PCR products

The DNA is then transcribed into RNA using T3 and T7 RNA polymerases together with fluorescently labelled UTP for incorporation into the growing chain of RNA. The reaction mixture comprises:

20µl 5X reaction buffer; 500µM ATP, CTP, GTP, fluorescent UTP (Amersham Ltd, UK); DEPC treated dH_2O; 1 unit T3 RNA polymerase or 1 unit T7 RNA polymerase (Promega Ltd., Southampton, UK); 1 unit Rnasin ribonuclaese inhibitor and DNA from PCR (1/3 of total, 10µl in dH_2O).

The mixture is incubated at 37°C for 1 hour. The mixture is then treated with DNAse to remove DNA so that only newly synthesised fluorescent RNA is left. The RNA is then precipitated, microcentrifuged and resuspended in buffer for hybridisation on the array.

c) Polymorphism Analysis

The sample-amplified fragments are then tested using a DNA microarray

The DNA microarray used comprises oligonucleotides which bind specifically to an allele of an amplified nucleic acid product as defined above. These oligonucleotides are applied by a robot onto a glass slide and immobilised. The fluorescently labelled amplified DNA is introduced onto the DNA microarray and a hybridisation reaction conducted to bind any complementary sequences in the sample, allowing unbound material to be washed away. The presence of bound samples is detected using a scanner. The absence of a fluorescent signal for a specific oligonucleotide probe indicates that the client does not have the corresponding polymorphism.

Example 5 DNA Array method for identifying A1298C polymorphism

The PCR reaction mix contains Taq polymerase (1.25 units/reaction), optimised PCR buffer, dNTP's (200µM each)

and $MgCl_2$ at an appropriate concentration of between 1 and 4 mM, and 40 pmol of each primer (SEQ ID NOs: 3 & 4) for amplification of the fragment. The methods used is the same as detailed in Example 4, with the array comprising the oligonucleotide SEQ ID NO: 55.

The presence of bound samples is detected using a scanner as described above. A highly fluorescent spot is detected at the positions corresponding to the oligonucleotides SEQ ID NO: 55. No signal is seen at the spots corresponding to SEQ ID NO: 56, demonstrating that the sample is not heterozygous for the wt allele.

Example 6 Generation of Report

The results of the microarray or Taqman® analysis for a sample having a particular barcode are inputted into a computer and linked with the personal details of the individual who supplied the sample, using the barcode as a reference.

The computer comprises a second dataset correlating the presence of individual alleles with a risk factor and a third dataset correlating risk factors with lifestyle advice. A report is generated identifying the presence of particular polymorphisms and providing lifestyle recommendations based on the identified polymorphisms and the personal details of the individual. An example of such a decision process is shown in Table 4.

A barcoded sample of DNA is screened and the alleles identified input to a data processor as Dataset 1 and matched with the subjects personal details using the barcode. Each allele is matched to lifestyle risk factor

from dataset 2. The identified risk factor is then matched with one or more lifestyle recommendations from dataset 3, for example "avoid red meat, chargrilled food, smoked meats and fish; stop smoking immediately" (in order to avoid production of potentially toxic byproducts by Phase 1 enzymes with increased activity) and "increase consumption of vegetables of the allium family e.g. onions and garlic, and the brassaicae family e.g. broccoli" (in order to increase the activity of Phase 11 enzymes present, such as GSTP1 and GSTT1 and others, in order to increase the excretion of toxic byproducts of Phase 1 metabolism). This is then checked against the subjects personal details in the data processor, e.g. age, sex, weight smoking status, exercise levels and existing diet to modify the recommendation accordingly before generating the final recommendation appropriate to the allele. The lifestyle recommendations are then assembled to generate a comprehensive personalised lifestyle advice plan.

Example 7 Presentation of Individual Results

The report document contains information from a number of sources (lifestyle, wellbeing factors, demographic and health data, and genetic information) and provides a clear set of advice which will help an individual optimise their health status.

The report consists of several sections; Your Personal Lifestyle Report; Your Genetic Results; Understanding Your Genes; Vitamin and Mineral Guide; FAQs and Quick Reference

The report as presented contains the key advice as an easy to read lifestyle summary (Table 5) and a summary of the genetic variations found (Table 6) in an easy to read

format. The clarity and ease of understanding of the summary is enhanced by the use of visual icons to symbolize the results (Table 7).

The advice is then shown as a series of statements covering each of the areas investigated (Table 5).

Once produced, the personalised sections of the report document are sealed using a paper wrapper, the document is placed in an envelope and then sent to the individual.

References

Abravaya, K., Carrino, J. J., Muldoon, S., and Lee, H. H. 1995. Detection of point mutation with a modified ligase chain reaction (Gap-LCR). Nucleic Acids Research. 23:675-682.

Agarwal, K., Jones, D. E., Daly, A. K., James, O. F., Vaidya, B., Pearce, S. & Bassendine, M. F., 2000, CTLA-4 gene polymorphism confers susceptibility to primary biliary cirrhosis, J Hepatol, 32, 4, p. 538 - 541.

Alexandrie, A.-K., Warholm, M., Carstensen, U., Axmon, A., Hagmar, L., Levin, J.O., Ostman, C., and Rannug, A. CYP1A1 and GSTM1 polymorphisms affect urinary 1-hydroyprene levels after PAH exposure. Carcinogenesis 21(4)669-676, 2000.

Ambrosone, C.B., Freudenheim, J.L., Thompson, P.A., Bowman, E., Vena, J.E., Marshall, J.R., Graham, S., Laughlin, R., Nemoto, T., and Shields P.G. Manganese Superoxide Dismutase (MnSOD) Genetic Polymorphisms, Dietary Antioxidants, and Risk of Breast Cancer. Cancer Research 59: 602-606, 1999.

Ames, B. N. Cancer prevention and diet: Help from single nucleotide polymorphisms. Proceedings of the National Academy of Science USA 96(22): 12216-12218, 1999.

Aron, Y., Swierczewski, E., Lockhart, A., 1994. A simple and rapid micromethod for genomic DNA extraction from jugal epithelial cells. Application to human lymphocyte antigen typing in one large family of atopic/asthmatic probands. Allergy 49 (9): 788-90.

Ban, Y. & Taniyama, M., 2000, Vitamin D Receptor Gene Polymorphism Is Associated with Graves' Disease in the Japanese Population, J Clin Endocrinol Metab, 85, 12, p. 4639-4643.

Barany, F. 1991. Genetic disease detection and DNA amplification and DNA amplification using cloned thermostable ligase. Proceedings of the National Academy of Science. USA 88:189-193.

Bell, D.A, Stephens, E., Castranio, T., Umback, D.M., Watson, M., Deakin, M., Elder, J., Duncan, H., Hendrickse, C., Strange, R.C. Polyadenylation polymorphism in the N-actetyltransferase gene 1 (NAT1) increases risk of colorectal cancer. Cancer Research 55: 3537-3542, 1995.

Bosron, W.F. and Li, T.K. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases and their relationship to alcohol metabolism and alcoholism. Hepatology 6: 502-510, 1986.

Brand, E., Ringel, J. & Sharma, A. M., 2000, Role of the angiotensinogen gene for essential hypertension, Herz, 25, 1, p. 15 - 25.

Breslauer, et al., "Predicting DNA duplex stability from base sequence", Proc. Nat'l Acad. Sci. USA, 83: 3746-3750 (1986)

Brockton, N., Little, J., Sharp, L, and Cotton, S.C. N-Acetyltransferase Polymorphisms and Colorectal Cancer: A HuGE Review. American Journal of Epidemiology 151(9): 846-861, 2000.

Bryant, M.S., Skipper, P.L., Tannenbaum, S.R., and Niure, M. Haemoglobin adducts of 4-aminobiphenyl in smokers and non-smokers. Cancer Research 47: 612-618, 1987.

Buervenich, S., Sydow, O., Carmine, A., Zhang, Z., Anvret, M. & Olson, L., 2000, Alcohol dehydrogenase alleles in Parkinson's disease, Mov Disord, 15, 5, p. 813-818.

Bullido, M. J. & Valdivieso, F., 2000, Apolipoprotein E gene promoter polymorphisms in Alzheimer's disease, Microsc Res Tech, 50, 4, p. 261 - 267.

Cheung, V. G., et. al., 1999, Nature, Genetics, vol. 21, 15-19.

Corbo, R. M. & Scacchi, R., 1999, Apolipoprotein E (APOE) allele distribution in the world. Is APOE*4 a 'thrifty' allele?, Ann Hum Genet, 63, PT4, p. 301 - 310.

Costa, L. G., 2000, The emerging field of ecogenetics, Neurotoxicology, 21, 1-2, p. 85-89.

Cotton, S.C., Sharp, L., Little, J., and Brockton, N. Glutathione S-Transferase Polymorphisms and Colorectal Cancer (A HuGE review). American Journal of Epidemiology 151(1)7-32, 2000.

Cramer, D. W., Greenberg, E. R., Titus-Ernstoff, L., Liberman, R. F., Welch, W. R., Li, E. & Ng, W. G., 2000, A case-control study of galactose consumption and metabolism in relation to ovarian cancer, Cancer Epidemiol Biomarkers Prev, 9, 1, p. 95 -101.

Cramer, D. W., Greenberg, E. R., Titus-Ernstoff, L., Liberman, R. F., Welch, W. R., Li, E. & Ng, W. G., 2000, A case-control study of galactose consumption and metabolism in relation to ovarian cancer, Cancer Epidemiol Biomarkers Prev, 9, 1, p. 95-101.

de Padua Mansur, A.; Annicchino-Bizzacchi, J.; Favarato, D.; Avakian, S. D.; Machado Cesar, L. A.; Franchini Ramires, J. A., 2000. Angiotensin-converting enzyme and apolipoprotein B polymorphisms in coronary artery disease. Am J Cardiol 85 (9): 1089-93.

Di Castelnuovo, A., D'Orazio, A., Amore, C., Falanga, A., Donati, M. B. & Iacoviello, L., 2000, The decanucleotide insertion/deletion polymorphism in the promoter region of the coagulation factor VII gene and the risk of familial myocardial infarction, Thromb Res, 98, 1, p. 9 - 17.

Dickey, C., Snatella, R., Hattis, D., Tang, D., Hsu, Y., Cooper, T., Young, T. and Perera F., Variability in PAH-DNA adduct measurements in peripheral mononuclear cells: implications for quantitative cancer risk assessment. Risk Analysis 17: 649-655, 1997.

Dietz, A.C., Zheng, W., Leff, M.A., Gross, M., Xiao, G.-F., Doll, M.A., Wen, W.-Q., Folsom, A.R., Hein, D.W. N-acetyltransferase-2 (NAT2) acetylation polymorphism, well-done meat intake and breast

cancer risk among post-menopausal women. Proceedings of the American Association for Cancer Research, 40: 148, 1999.

Doll, M.A., Jiang, W., Deitz, A.C., Rustan, T.D., and Hein, D.W. Identification of a novel allele at the human NAT1 acetyltransferase locus. Biochem. Buiophys. Res. Commun. 233: 584-591, 1997.

Donati, M. B., Zito, F., Castelnuovo, A. D. & Iacoviello, L., 2000, Genes, coagulation and cardiovascular risk, J Hum Hypertens, 14, 6, p. 369 - 372.

Donis-Keller H., Green P, Helms C., et. al. (1987), A genetic map of the human genome. Cell, 51, 319-337

Eberhart, M.V., Lee, C.Y., Liu, R.H. Antioxidant activity of fresh apples. Nature 405: 903-904, 2000.

Fernandez-Real, J. M., Vendrell, J., Ricart, W., Broch, M., Gutierrez, C.,

Casamitjana, R., Oriola, J. & Richart, C., 2000, Polymorphism of the tumor necrosis factor-alpha receptor 2 gene is associated with obesity, leptin levels, and insulin resistance in young subjects and diet-treated type 2 diabetic patients, Diabetes Care, 23, 6, p. 831-837.

Garte, S. The role of ethnicity in cancer susceptibility gene polymorphisms: the example of CYP1A1. Carcinogenesis 19(8) 1329-1332, 1998.

Gelder, C. M., Hart, K. W., Williams, O. M., Lyons, E., Welsh, K. I., Campbell, I. A., Marshall, S. E., 2000, Vitamin D receptor gene polymorphisms and susceptibility to Mycobacterium malmoense pulmonary disease, J Infect Dis, 181, 6, p. 2099-2102.

Gibbs, R. A., Nguyen, P. N., and Caskey, C. T. 1989. Detection of single DNA base differences by competitive oligonucleotides priming. Nucleic Acids Research. 17:2437-2448.

Gil, J.P., Lechner, M.C. Increased frequency of wild type arylamine-N-actyltransferase allel NAT2*4 homozygotes in Portuguese patients with colorectal cancer. Carcinogenesis 19(1) 37-41, 1998.

Giovannucci, E. Nutritional factors in human cancers. Advances in Experimental Medicine and Biology 472:29-42, 1999.

Grossman, P. D., Bloch, W., Brinson, E., Chang, C. C., Eggerding, F. A.,

Fung, S., Iovannisci, D. A., Woo, S., and Winn-Deen, E. S. 1994. High-density multiplex detection of nucleic acid sequences: oligonucleotides ligation assay and sequence-coded separation. Nucleic Acid Research. 22:4527-4534.

Harries, L.W., Stubbins, M.J., Forman, D., Howard, G.c.W, Wolf R. Identification of genetic polymorphisms at the glutathione S-transferase pi locus and association with susceptibility to bladder, testicular, and prostate cancer. Carcinogenesis 18:641-644, 1997.

Hattis D., Erdreich, L, and DiMauro, T. Human Variability in Parameters that are Potentially Related to Susceptibility to Carcinogenesis-I. Preliminary Observations. Center for Technology, Policy and Industrial Development, MIT, Cambridge, MA., 1986.

Heilbronn, L. K., Noakes, M., Morris, A. M., Kind, K. L., Clifton, P. M., 2000, 360His polymorphism of the apolipoproteinA-IV gene and plasma lipid response to energy restricted diets in overweight subjects, Atherosclerosis, 150, 1, p. 187 - 192.

Hein, D., Doll, M.A., Fretland, A.J., Leff, M.A., Webb, S.J., Xiao, U.-S.D., Nangju, N., Feng, Y., Molecular Genetics and Epidemiology of the NAT1 and NAT2 Acetylation Polymorphisms. Cancer Epidemiology, Biomarkers & Prevention 9: 29-42, 2000 (a).

Hein, D., N-Acetyltransferase genetics and their role in predisposition to aromatic and heterocyclic amine-induced carcinogenesis. Toxicology Letters 112-113: 349-356, 2000 (b).

Hennig, B. J., Parkhill, J. M., Chapple, I. L., Heasman, P. A. & Taylor, J. J., 1999, Association of a vitamin D receptor gene polymorphism with localized early-onset periodontal diseases, J Periodontol, 70, 9, p. 1032-1038.

Hirvonen, A. Polymorphisms of Xeno-biotic-Metaboilzing Enzymes and Susceptibility to Cancer. Environ Health Perspect 107 Supplement 1: 37-47, 1999.

Humphries, S. E., Henry, J. A. & Montgomery, H. E., 1999, Gene-environment interaction in the determination of levels of haemostatic variables involved in thrombosis and fibrinolysis, Blood Coagul Fibrinolysis, 10 Suppl 1, p. S17 - S21.

Ikuta, S., Takagi K., Wallace, R. B., and Itakura, K. 1987. Dissociation Kinetics of 19 base paired oligonucleotides-DNA Duplexes containing different single mismatched base pairs. Nucleic Acids Research. 15:797-811.

Ilett, K.F., David, B.M., Dethon, P., Castlden, W.M, and Kwa, R. Acetylation pehotype in colorectoal carcinonoma. Cancer Research 47: 1466-1469, 1987.

International Agency for Research on Cancer (IARC). Alcohol Drinking. IARC monographs on the evaluation of the carcinogenic risks to humans, IARC, Lyon. 44: 153-246, 1998.

Kato, S., Bowman, E.D., Harrington, A.M., et al Human lung carcinogen DNA adduct levels mediated by genetic polymorphisms in vivo. Journal of the National Cancer Institute 87:902-907, 1995.

Kawajiri, K., Eguchi, H., Nakachi, K., Sekiya., T., Yamamoto, M. Association of CYP1A1 germ line polymorphisms with mutations of the p53 gene in lung cancer. Cancer Research 56:72-76, 1996.

Landegren, U., Kaiser, R., Sanders, J., and Hood, L, 1988. A ligand-mediated gene detection technique. Science. 241:1077-1080.

Laplaud, P. M., Dantoine, T. & Chapman, M. J., 1998, Paraoxonase as a risk marker for cardiovascular disease: facts and hypotheses, Clin Chem Lab Med, 36, 7, p. 431-441.

Layton, D.W., Bogen, K.T., Knize, M.G., Hatch, F.T., Johnson, V.M., and Felton, J.S. Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. Carcinogenesis 16: 39-52, 1995.

Lee, E., Huang, Y., Zhao, B. et al Genetic polymorphism of conjugating enzymes and cancer risk: GSTM1, GSTT1, NAT1 and NAT2. Journal of the Toxicological Society 23: 140-142, 1998.

Maciag, P. C., Schlecht, N. F., Souza, P. S., Franco, E. L., Villa, L. L. & Petzl-Erler, M. L., 2000, Major histocompatibility complex class II polymorphisms and risk of cervical cancer and human papillomavirus infection in Brazilian women, Cancer Epidemiol Biomarkers Prev, 9, 11, p. 1183 - 1191.

MacKness, B., Mackness, M. I., Durrington, P. N., Arrol, S., Evans, A. E., McMaster, D., Ferrieres, J., Ruidavets, J. B., Williams, N. R. & Howard, A. N., 2000, Paraoxonase activity in two healthy populations with differing rates of coronary heart disease, Eur J Clin Invest, 30, 1, p. 4 - 10.

MacLeod, S., Sinha, R., Kadlubar, F.F., Lang, N.P. Polymorphisms of CYP1A1 and GSTM1 influence the in vivo function of CYP1A2. Mutation Research 376(1-2): 135-142, 1997.

Maniatis T., Fritsch E. F., and Sambrook J., (1982) Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Springs Harbor, NY.

Marchand, L. L., Wilkinson, G. R. & Wilkens, L. R., 1999, Genetic and dietary predictors of CYP2E1 activity: a phenotyping study in Hawaii Japanese using chlorzoxazone, Cancer Epidemiol Biomarkers Prev, 8, 6, p. 495 - 500.

Matthias, C., Bockmuhl, U., Jahnke, V., Harries, L., Wolf, C.R., Jones, P.W., Alldersea, J. Worrall, S.F., Hand, P., Fryer, A.A. et al, The glutathione -S-transferase GSTP1 polymorphism: effects on susceptibility to oral/phryngeal and laryngeal carcinomas. Pharmacogenetics 8: 1-6, 1997.

Miki, M. & Satoh, K., 1999, Genetic risk factors for chronic obstructive pulmonary disease (COPD), Nippon Rinsho, 57, 9, p. 1954 - 1958.

Mooney, L.A., Perera, F.P. Application of molecular epidemiology to lung cancer chemoprevention. Journal of Cellular Biochemistry Supplement 25:63-8, 1996.

Mooney, L.A., Santella, R.M., Covey, L., Jeffrey, A.M., Bigbee, W., Randall, M.C., Cooper, T.B., Ottman, R., Tsai, W.-Y., Wazneh, L. et al. Decline in DNA damage and other biomarkers in peripheral blood following smoking cessation. Cancer Epidemiological Biomarkers Prevention 4: 627-634, 1995.

Nakajima, T. & Aoyama, T., 2000, Polymorphism of drug-metabolizing enzymes in relation to individual susceptibility to industrial chemicals, Ind Health, 38, 2, p. 143 - 152.

Newton, C. R., Graham, A., Heptinstall, L. E., Powell, S. J., Summers, C., Kalsheker, N., Smith, J. C., and Markham, A. F. 1989. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Research. 17:2503-2516.

Nickerson, D. A., Kaiser, R., Lappin, S., Stewart, J., Hood, L., and Landegren, U. 1990. Automated DNA diagnostics using an ELISA-based oligonucleotides ligation assay. Proceedings of the National Academy of Science. USA 87:8923-8927.

Oliveira, J. R. & Zatz, M., 1999, The study of genetic polymorphisms related to serotonin in Alzheimer's disease: a new perspective in a heterogenic disorder, Braz J Med Biol Res, 32, 4, p. 463-467.

Ordovas, J. M., Cupples, L. A., Corella, D., Otvos, J. D., Osgood, D., Martinez, A., Lahoz, C., Coltell, O., Wilson, P. W., Schaefer, E. J., 2000, Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study, Arterioscler Thromb Vasc Biol, 20, 5, p. 1323-1329.

Ota, N., Hunt, S. C., Nakajima, T., Suzuki, T., Hosoi, T., Orimo, H., Shirai, Y. & Emi, M., 1999, Linkage of interleukin 6 locus to human osteopenia by sibling pair analysis, Hum Genet, 105, 3, p. 253-257.

Perera, F. P. Molecular epidemiology and prevention of cancer. Environmental Health Perspectives 103 Suppl 8: 233-6, 1995.

Perera, F.P. Biomarkers and Molecular Epidemiology of Cancer. Proceedings of the 9th International Symposium in Epidemiology in Occupational Health. National Institute for Occupational Safety and Health, Cincinnati, OH. PP 54-66, 1992.

Perera, F.P. Environment and cancer: Who are susceptible? Science 278: 1068-1073, 1997.

Perera, F.P. and Weinstein I.B. Molecular epidemiology: recent advances and future directions. Carcinogenesis 21 (3): 517-524, 2000.

Pfutzer, R. H., Barmada, M. M., Brunskill, A. P., Finch, R., Hart, P. S., Neoptolemos, J., Furey, W. F. & Whitcomb, D. C., 2000, SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis, Gastroenterology, 119, 3, p. 615 - 623.

PicoGreen dsDNA Quantitation Reagent and Kit Instruction, (1996) Molecular Probes, Eugene, Or.

Pluth, J. M., Nelson, D. O., Ramsey, M. J. & Tucker, J. D., 2000, The relationship between genotype and chromosome aberration frequencies in a normal adult population, Pharmacogenetics, 10, 4, p. 311 - 319.

Poolsup, N., Li Wan Po, A. & Knight, T. L., 2000, Pharmacogenetics and psychopharmacotherapy, J Clin Pharm Ther, 25, 3, p. 197 - 220.

Potter, J. D. Colorectal cancer: Molecules and Populations. Journal of the National Cancer Institute 91(11): 916-932, 1999.

Raknes, G., Fernandes Filho, J. A., Pandey, J. P., Myhr, K. M., Ulvestad, E., Nyland, H., Vedeler, C. A., 2000, IgG allotypes and subclasses in Norwegian patients with multiple sclerosis, J Neurol Sci, 175, 2, p. 111 - 115.

Rojas, M., Cascorbi, I., Alexandrov, K., Kried, E., Auburtin, G., Mayer, L., Kopp-Schnieder, A., Roots, I., and Bartsch, H. Modulation of benzo[a]pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1 GSTM1 and GSTT1 polymorphism, Carcinogenesis 21(1): 35-41, 2000.

Rosa-Rosa, L., Zimmermann, N., Bernstein, J. A., Rothenberg, M. E. & Khurana Hershey, G. K., 1999, The R576 IL-4 receptor alpha allele correlates with asthma severity, J Allergy Clin Immunol, 104, 5, p. 1008-1014.

Ryberg, D., Skaug, V., Hewer, A., Phillips, D.H., Harries, L.W., Wolf, C.R., Ogreid, D., Ulvik, A., Vu, P. Haugen, A. Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. Carcinogenesis 18:1285-1289, 1997.

Rylchik, W., "Selection of Primers for Polymerase Chain Reaction", Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications, pp 31-40 (1993) Humana Press.

Saiki, R. K., Walsh, P. S., Levenson, C. H., and Erlich, H. A. 1989. Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotides probes. Proceedings of the National Academy of Science. USA 86:6230-6234.

Salas, J., Jansen, S., Lopez-Miranda, J., Ordovas, J. M., Castro, P., Marin, C., Ostos, M. A., Bravo, M. D., Jimenez-Pereperez, J., Blanco, A., Lopez-Segura, F., Perez-Jimenez, F., 1998, The SstI polymorphism of the apolipoprotein C-III gene determines the insulin response to an

oral-glucose-tolerance test after consumption of a diet rich in saturated fats, Am J Clin Nutr, 68, 2, p. 396-401.

Schabath, M. B., Spitz, M. R., Zhang, X., Delclos, G. L. & Wu, X., 2000, Genetic variants of myeloperoxidase and lung cancer risk, Carcinogenesis, 21, 6, p. 1163 - 1166.

Schena, M., 1999, DNA Microarrays "a practical approach", ISBN, 0-19-963777-6, Oxford press, editor B. D. Hames

Shields, P. G., Harris, C.C. Cancer Risk and low-Penetrance Susceptibility Genes in Gene-Environment Interactions. Journal of Clinical Oncology 18(11) 2309-2315, 2000.

Sinha, R. & Caporaso, N., 1997, Heterocyclic amines, cytochrome P4501A2, and N-acetyltransferase: issues involved in incorporating putative genetic susceptibility markers into epidemiological studies, Ann Epidemiol, 7, 5, p. 350 - 356.

Sinha, R., Chow,W.H., Kulldorff, M., Denobile, J., Butler, J., Garcia-Closas, M., Weil, R., Hoover, R.N., and Rothman, N. Well-done, Grilled Red Meat Increases the Risk of Colorectal Adenomas. Cancer Research 59: 4320-4324, 1999.

Smith, G., Stanley, L.A., Sim, E., Strange, R., and Wolf, C.R. Metabolic Polymorphisms and Cancer Susceptibility. Cancer Surveys 25: 27-65, 1995.

Syvanen, A. C., Sayantile, A., and Lukka, M. 1993. Identification of individuals by analysis of biallelic DNA markers, Using PCR and solid-phase minisequencing. American Journal of Human Genetics. 52:46-59.

Taningher, M., Malacarne, D., Izzotti, A., Ugolini, D. Parodi, S. Drug metabolism polymorphisms as modulators of cancer susceptibility. Mutation Research 436: 227-261, 1999.

Togo, A. V., Suspitsin, E. N., Grigoriev, M. Y., Ilyushik, E. S., Karpova, M. B., Hanson, K. P. & Imyanitov, E. N., 2000, L-myc

polymorphism in cancer patients, healthy blood donors and elderly, tumor-free individuals in Russia, Int J Cancer, 85, 6, p. 747-750.

Tsai, M. Y., Welge, B. G., Hanson, N. Q., Bignell, M. K., Vessey, J., Schwichtenberg, K., Yang, F., Bullemer, F. E., Rasmussen, R. & Graham, K. J., 1999, Genetic causes of mild hyperhomocysteinemia in patients with premature occlusive coronary artery diseases, Atherosclerosis, 143, 1, p. 163-170.

Ulrich, C.M., Kampman, F., Bigler, J., Schwartz, S.M., Chen, C., Bostick, R., Fosdick, L., Bereford, S.A.A., Yasui, Y., and Potter, J.D. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? Cancer Epidemiological Biomarkers Prevention 8: 659-668, 1999.

Verlaan-de Vries, M., Bogaard, M. E., van den Elst, H., van Boom, J. H., van der Eb, A. J., and Bos, J. L. 1986. A dot-blot screening procedure for mutated ras oncogenes using synthetic oligodeoxynucleotides. Gene. 50:313-320.

Vicente, V., Gonzalez-Conejero, R., Rivera, J. & Corral, J., 1999, The prothrombin gene variant 20210A in venous and arterial thromboembolism, Haematologica, 84, 4, p 356 - 362.

Vineis, P. Molecular Epidemiology: Low-dose Carcinogens and genetic susceptibility. International Journal of Cancer 71: 1-3, 1997.
Wallace, A. J., Humphries, S. E., Fisher, R. M., Mann, J. I., Chisholm, A., Sutherland, W. H., 2000, Genetic factors associated with response of LDL subfractions to change in the nature of dietary fat, Atherosclerosis, 149, 2, p 387 - 394.

Wallace, R. B., Johnson, M. J., Hirose, T., Miyake, T., Kawashima, E. H. and Itakura, K., 1981. The use of synthetic oligonucleotides as hybridisation probes. II. Hybridization of oligonucleotides of mixed sequence to rabbit beta-globin DNA. Nucleic Acids Research. 9:879-894.

Wilkinson, R. J., Llewelyn, M., Toossi, Z., Patel, P., Pasvol, G., Lalvani, A., Wright, D., Latif, M. & Davidson, R. N., 2000, Influence of vitamin D deficiency and vitamin D receptor polymorphisms on

tuberculosis among Gujarati Asians in west London: a case-control study, Lancet, 355, 9204, p. 618-621.

World Cancer Research Fund (WCRF) Panel. (Potter, J.D. Chair) Diet, nutrition, and the prevention of cancer: a global perspective. Washington, D.C.: WCRF/American Institute of Cancer Research, 1997.

Wu, D. Y., and Wallace, R. B. 1989. The ligation amplification reaction (LAR)-amplification of specific DNA sequences using sequential rounds of template-dependent ligation. Genomics. 4:560-569.

Yamada, N., Yamaya, M., Okinaga, S., Nakàyama, K., Sekizawa, K., Shibahara, S. & Sasaki, H., 2000, Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema, Am J Hum Genet, 66, 1, p. 187 - 195.

Yershov, G., Barsky, V., et. al., 1996, Proc. Natl. Acad. Sci. USA, Genetics, Vol. 93, 4913-4918.

Yokota, M., Ichihara, S., Lin, T. L., Nakashima, N. & Yamada, Y., 2000, Association of a T29-->C polymorphism of the transforming growth factor- beta1 gene with genetic susceptibility to myocardial infarction in Japanese, Circulation, 101, 24, p. 2783-2787.

Yokoyama, A., Muramatsu, T., Ohmori, T., Yokoyama, T., Okuyama, K., Takahashi, H., Hasegawa, Y., Higuchi, S., Maruyama, K., Shirakura, K., Ishii, H. Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. Carcinogenesis 19(8)1383-1387, 1998.

Zhang, Y., Coyne, M.Y., Will, S. G., Levenson, C. H., and Kawasaki, E. S. 1991. Single-base mutational analysis of cancer and genetic disease using membrane bound modified oligonucleotides. Nucleic Acids Research. 19: 3929-3933.

Zheng, W., Deitz, A.C., Campbell, D.R., Wen, W-Q., Cerhan, J.R., Sellers, T.A., Folsom, A.R., and Hein, D.W. N-acetyltransferase I genetic polymorphism, cigarette smoking, well-done meat intake, and breast cancer risk. Cancer Epidemiological Biomarkers Prevention 8: 233-239, 1999.

Zychma, M. J., Gumprecht, J., Zukowska-Szczechowska, E. & Grzeszczak, W., 1999, Polymorphisms in the genes encoding for human kinin receptors and the risk of end-stage renal failure: results of transmission/disequilibrium test. The End-Stage Renal Disease Study Group, J Am Soc Nephrol, 10, 10, p. 2120-212

GENE	SNP	WT 1	MUT 2	PCR 1	PCR 2
MTHFR	MTHFR C677T	C.	T	AGCGGATAACATGCCTTCACAAAGCGGAAG (SEQ ID NO:1)	AGCGGATAACCTTGAAGGAGAAGGTGTCTG(SEQ ID NO:2)
	MTHFR A1298C	A	C	AGCGGATAACAAGAGCAAGTCCCCCAAGGA (SEQ ID NO:3)	AGCGGATAACCTTTGTGACCATTCCGGTTTG(SEQ ID NO:4)
MTR	MTR A2756G	A	G	AGCGGATAACATCTCCTCTCCTTGAGAGAC(SEQ ID NO:5)	AGCGGATAACGTGTTCCCAGCTGTTAGATG(SEQ ID NO:6)
MTRR	MTRR A66G	A	G	AGCGGATAACATATGCTACACAGCAGGGAC(SEQ ID NO:7)	AGCGGATAACGAAAATCCATGTACCACAGC(SEQ ID NO:8)
CBS	CBS C699T	C	T	AGCGGATAACTCCTAGACCAGTACCGCAAC(SEQ ID NO:9)	AGCGGATAACTGCTGCAGGATCTCATCAGC(SEQ ID NO:10)
VDR	VDR Taq1	C	T	AGCGGATAACTGTACGTCTGCAGTGTGTTG(SEQ ID NO:11)	AGCGGATAACTGTGCCTTCTTCTCTATCCC(SEQ ID NO:12)
	VDR Bsm1	T	C	AGCGGATAACTAGATAAGCAGGGTTCCTGG(SEQ ID NO:13)	AGCGGATAACAGTTCACGCAAGAGCAGAGC(SEQ ID NO:14)
	VDR Apa1	T	G		
	VDR Fok 1	T	C	TCAAAGTCTCCAGGGTCAGG(SEQ ID NO:15)	TGGCCTGCTTGCTGTTCTTA(SEQ ID NO:16)
COL1A1	COL1A1 G Sp1T	G	T	AGCGGATAACAGGAGAGAAGGGAAGGTCCAG(SEQ ID NO:17)	AGCGGATAACAATCAGCCGCTCCCATTCTC(SEQ ID NO:18)
IL6	IL-6 G634C	G	C	AGCGGATAACGAGTTCTTGCTGTGTTCTGG(SEQ ID NO:19)	AGCGGATAACCTGCACGAAATTTGAGGGTG(SEQ ID NO:20)
	IL-6 G174C	G	C	AGCGGATAACGATTGTGCAATGTGACGTCC(SEQ ID NO:21)	AGCGGATAACAGCCTCAATGACGACCTAAG(SEQ ID NO:22)
TNFa	TNF G-308A	G	A	AGCGGATAACGATTTGTGTGTAGGACCCTG(SEQ ID NO:23)	AGCGGATAACGGTCCCCAAAAGAAATGGAG(SEQ ID NO:24)
ACE	ACE II/DD	Insertion	Deletion	AGCGGATAACctggagaccactcccatcctttct(SEQ ID NO:25)	AGCGGATAACgatgtggccatcacattggtcagat(SEQ ID NO:26)
PPAR	PPAR Pro12Ala	C	G	AGCGCAA GTCTTTTTCTTTTAACGGATTGATC(SEQ ID NO:27)	AGGATACCC AATAGCCGTATCTGGAAGGAA (SEQ ID NO:28
SOD2	SOD2 C-28T	C	T	AGCGGATAACTTCTGCCTGGAGCCCAGATAC(SEQ ID NO:29)	AGCGGATAACTTCTCGTCTTCAGCACCAGC(SEQ ID NO:30)
	SOD2 T175C	T	C	AGCGGATAACACCATTGAACTTCAGTGCAG(SEQ ID NO:31)	AGCGGATAACCAGTGGTTGAAAAAGTAGGAG(SEQ ID NO:32)
SOD3	SOD3 C760G	C	G	AGCGGATAACAGGCGCGGGAGCACTCAGAG(SEQ ID NO:33)	AGCGGATAACGCCTTGCACTCGCTCTCGC(SEQ ID NO:34)
GSTM1	GSTM1	Present	deletion	AGCGGATAACGAAGGTGGCCTCCTCCTTGG(SEQ ID NO:35)	AGCGGATAACGCCCAGCTGCATATGGTTGT(SEQ ID NO:36)
GSTT1	GSTT1	Present	Deletion	AGCGGATAACTTCCTTACTGGTCCTCACATCTC(SEQ ID NO:37)	AGCGGATAACTCACCGGATCATGGCCAGCA(SEQ ID NO:38)
GSTP1	GSTP1 A313G	A	G	AGCGGATAACACATGGTGAATGACGGCGTG(SEQ ID NO:39)	AGCGGATAACGCAGATGCTCACATAGTTGG(SEQ ID NO:40)
	GSTP1 C341T	C	T	AGCGGATAACCTCAAAAGGCTTCAGTTGCC(SEQ ID NO:41)	AGCGGATAACTGATACATGGTGGTGTCTGG(SEQ ID NO:42)
APOC3	APOC3 C3175G	C	G	AGCGGATAACAAGCCCTGGAGATTGCAGGAC(SEQ ID NO:43)	AGCGGATAACAATACCCCAAGTCCACCTGC(SEQ ID NO:44)
CETP	CETP G279A	G	A	AGCGGATAACTTTGTCTGCGACCCAGAATC(SEQ ID NO:45)	AGCGGATAACACATTAACCCCCTAACCTGG(SEQ ID NO:46)
LPL	LPL C1595G	C	G	AAAGGCACCTGCGGTATTTG(SEQ ID NO:47)	AGCGGATAACCTTTAGCCCAGAATGCTCAC(SEQ ID NO:48)
eNOS	eNOS G894T	G	T	AGCGGATAACTGCTGCCCCTGCTGCTGCAG(SEQ ID NO:49)	AGCGGATAACACCTCAAGGACCAGCTCGG(SEQ ID NO:50)
F5	F5 G1691A	G	A	AGCGGATAACACCTAACATGTTCTAGCCAG(SEQ ID NO:51)	AGCGGATAACAATCTGTAAGAGCAGATCCC(SEQ ID NO52)
CBS	CBS T1080C	T	C	AGCGGATAACAGTGCTGGCAGCACGGTGG(SEQ ID NO:53)	AGCGGATAACAGAATGACCACGCAGCGCTG(SEQ ID NO:54)

Table 1

Gene	WT Probe	Polymorphism probe
GSTM1		
C534G	CAAGCAgTTGGGC (SEQ ID NO:58)	CAAGCAcTTGGGC (SEQ ID NO:57)
GSTP1		
A313G	GCAAATACaTCTCCCT (SEQ ID NO:60)	GCAAATACgTCTCCCT (SEQ ID NO:59)
C341T	CCTTGCCCgCCTC (SEQ ID NO:62)	CTTGCCCaCCTCC (SEQ ID NO:61)
GSTT1	CCTGCAGACCCC (SEQ ID NO:63)	N/A
MnSOD		
T-28C	ACCCCAAAaCCGGA (SEQ ID NO:65)	ACCCCAAAgCCGGA (SEQ ID NO:64)
T175C	AGCCCAGAtAGCT (SEQ ID NO:67)	AGCCCAGAcAGCT (SEQ ID NO:66)
MTHFR		
C677T	AAATCGgCTCCCGC (SEQ ID NO:69)	AAATCGaCTCCCGCAGA (SEQ ID NO:68)
C677T	AAATCGgCTCCCGC (SEQ ID NO:71)	AAATCGaCTCCCGCAGA (SEQ ID NO:70)
A1298C	CAGTGAAGaAAGTGTC (SEQ ID NO:56)	AGTGAAGcAAGTGTC (SEQ ID NO:55)

Table 2

Table 3

DATA SET 1												DATA SET 2	
							Genetic Susceptability ranking						
General Gene Marker Type	Examples of Gene Types	Gene Marker	Polymorphisms	Links with Cancer susceptabilities	Links with higher risks of cancer susceptibility	Homozygote or heterozygote relative to wild type	Reduces susceptibility	Normal susceptibility	Moderate Increase in susceptibility	Higher susceptibility	Very high susceptibility	Foods and other materials to avoid	Dietary and lifestyle advice
Type I		CYP											
	Genes that code for enzymes responsible for the detoxification of xenobiotics in Phase I metabolism		Cyp1A1-A (Wild-type)	Colorectal, urinary bladder, breast, oral cavity, stomach, and lung cancers		Homozygote		YES				Reduce consumption of sources of Xenobiotics (e.g. PAH) found in, for example, char-grilled red meat and smoked fish.	Consume food products, such as for example vegetables and fruit, e.g. cruciferous vegetables and allium family of vegetables.
			Cyp1A1-C	Colorectal, urinary bladder, breast, oral cavity, stomach, and lung cancers.		Homozygote				YES		Avoid consumption of sources of Xenobiotics (e.g. PAH) found in, for example, char-grilled red meat and smoked fish.	Increase consumption of food products known to induce Phase II metabolism, e.g. cruciferous and allium family vegetables.
						Heterozygotes			YES			Avoid consumption of sources of Xenobiotics (e.g. PAH) found in, for example, char-grilled red meat and smoked fish.	Increase consumption of food products known to induce Phase II metabolism, e.g. cruciferous and allium family vegetables.
			Ile-Val polymorphism	Colorectal, urinary bladder, breast, oral cavity, stomach and lung cancers.		Homozygote				YES		Avoid consumption of sources of Xenobiotics (e.g. PAH) found in, for example, char-grilled red meat and smoked fish.	Increase consumption of food products known to induce Phase II metabolism, e.g. cruciferous and allium family vegetables.
						Heterozygotes			YES			Avoid consumption of sources of Xenobiotics (e.g. PAH) found in, for example, char-grilled red meat and smoked fish.	Increase consumption of food products known to induce Phase II metabolism, e.g. cruciferous and allium family vegetables.
		NAT1											
			NAT1*4 (wild type)			Homozygote		YES				Reduce consumption of potential procarcinogens (e.g. PAH) found in, for example, char-grilled red meat and smoked fish.	Consume food products, such as for example vegetables and fruit, e.g. cruciferous and allium family of vegetables.
			NAT1*10	Colon cancer		Homozygote				YES		Avoid consumption of sources of Xenobiotics (e.g. PAH) found in, for example, char-grilled red meat and smoked fish.	Increase consumption of food products known to induce Phase II metabolism, e.g. Increase consumption of fruit and vegetables, particularly cruciferous vegetables such as broccoli and members of the allium family such as garlic and onion.

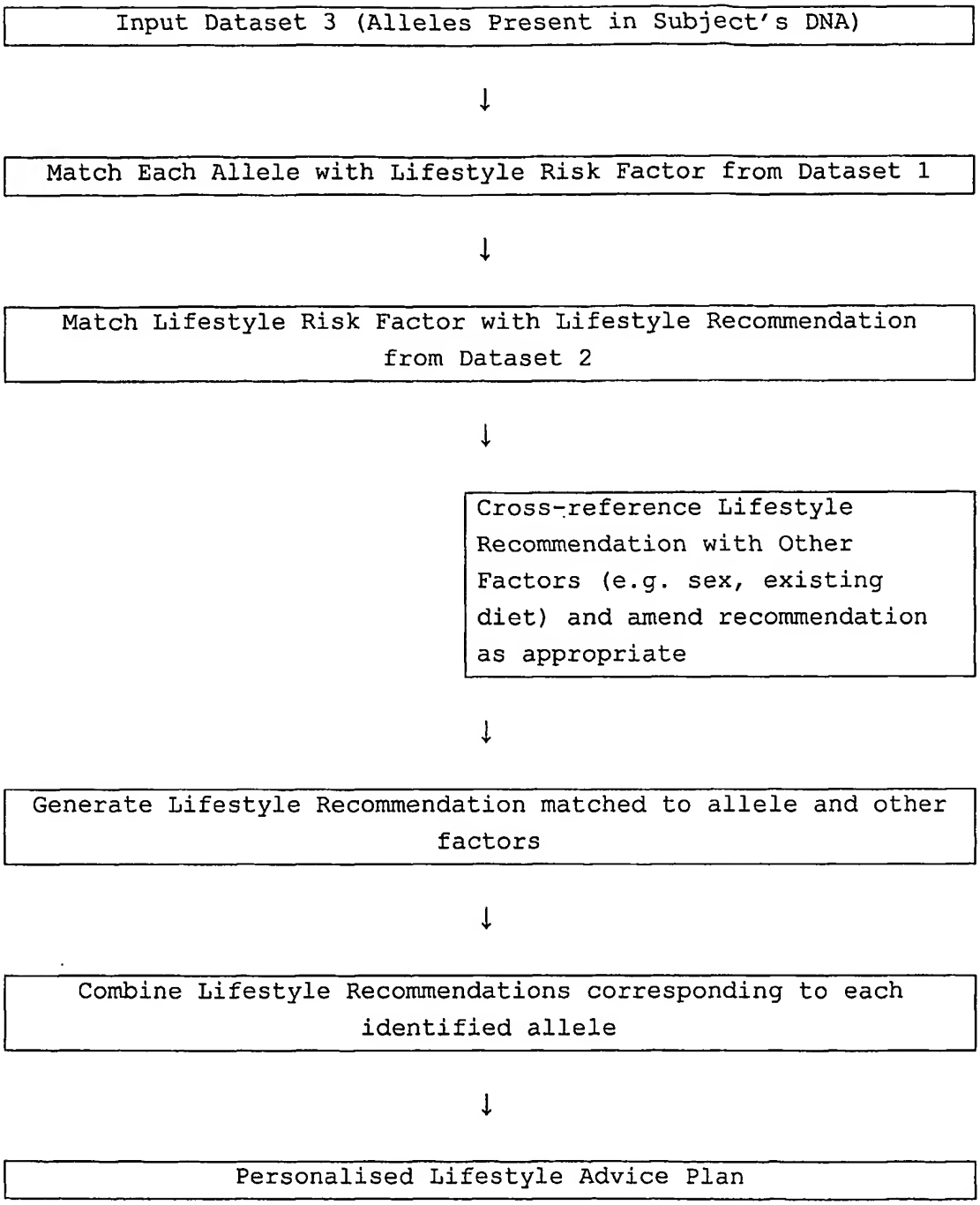
Input Dataset 3 (Alleles Present in Subject's DNA)
Match Each Allele with Lifestyle Risk Factor from Dataset 1
Match Lifestyle Risk Factor with Lifestyle Recommendation from Dataset 2
Cross-reference Lifestyle Recommendation with Other Factors (e.g. sex, existing diet) and amend recommendation as appropriate
Generate Lifestyle Recommendation matched to allele and other factors
Combine Lifestyle Recommendations corresponding to each identified allele
Personalised Lifestyle Advice Plan

Table 4

Dietary/Lifestyle Factors	*Our advice*
Folate	*Increase intake of folate as variation in your genetic profile has been found*
Vitamin B6	*Increase intake of vitamin B6 on account of variation in your genetic profile*
Vitamin B12	*Increase intake of vitamin B12 in view of your personal profile*
Fruit and vegetables	*Eat more fruits and vegetables to complement your personal profile*
Cruciferous vegetables	*Add these vegetables to your diet to complement your genetic profile*
Allium vegetables	*Add these vegetables to your diet in light of your personal profile*
Antioxidants	*Eat more antioxidants to complement your personal profile*
Calcium	*Good! Keep to current levels of calcium in your diet*
Vitamin D	*Good! Your consumption of vitamin D is optimal, keep it up*
Caffeine	*You currently consume very high amounts of caffeine.*
Refined carbohydrates	*Reduce your intake of refined carbohydrates to complement your profile*
Saturated fats	*Reduce saturated fats in your diet to complement your genetic profile*
Cholesterol	*Cut down on foods high in cholesterol as variation in your profile has been found*
Omega-3 fatty acids	*Your current intake of omega-3 fatty acids is good*
Tobacco	*Your choice not to smoke complements your personal profile*
Body weight	*Your BMI places you in your ideal weight range*
Physical activity	*Increase your level of physical activity to complement your profile*

Table 5

Gene Name	Area of Activity	How Common Are the Variations?	Your Genetic Profile
MTHFR	B vitamin Use/Heart Health	54 - 58%	Variation found
MS_MTRR	B vitamin Use/Heart Health	79%	Variation found
MTR	B vitamin Use/Heart Health	97%	Variation found
CBS	B vitamin Use/Heart Health	30 - 42%	Variation found
MnSOD	Antioxidants/Heart Health	20 - 50%	Variation found
SOD3	Antioxidants/Heart Health	3%	Variation found
GSTM1	Antioxidants/Detoxification	50%	Variation found
GSTT1	Antioxidants/Detoxification	15%	Variation found
GSTP1	Antioxidants/Detoxification	59%	Variation found
IL-6	Heart Health/Inflammation	68 - 82%	Variation found
APOC3	Heart Health	26%	Variation found
CETP	Heart Health	33%	Variation found
LPL	Heart Health	81%	Variation found
ACE	Heart Health/Insulin Sensitivity	36%	Variation found
VDR	Bone Health/Insulin Sensitivity	15 - 22%	Variation found
VDR Bsm	Bone Health/Insulin Sensitivity	35%	Variation found
COL1A1	Bone Health	10%	Variation found
PPAR-c2	Insulin Sensitivity	94%	Variation found
TNF-a	Inflammation/Bone Health/Insulin Sensitivity	6 – 43%	Variation found
Enos	Heart Health	54%	Variation found

Table 6

Your Personal Results At A Glance

Guide to symbols:

☺ Your lifestyle choices are excellent in this region	😐 These areas need a little consideration from you	☹ You need to pay particular attention to these areas	❖ You did not return your questionnaire/answer the relevant question(s)

Personal Lifestyle Results for Mr E.Ovlife

😐 Red meat – your consumption of well-done red meat is high, particularly in light of your genetic profile

☺ Smoked meat & fish – your eating habits are healthy but your genetic profile requires attention in this area

😐 Tobacco – your decision to stop smoking has benefited your health, particularly in light of your genetic profile

☹ Cruciferous vegetables (e.g broccoli, cauliflower) – increase your consumption of cruciferous vegetables as we have found a variation in your detoxification genes

☹ Allium vegetables (e.g onions, garlic) – increase your consumption of allium vegetables as we have found a variation in your detoxification genes

😐 Antioxidants – your current consumption of antioxidants is low and your genetic profile requires attention in this area

☹ Folate – you need to increase your current low intake of folate, B6 and B12, as we have found a variation in a key gene involved in B vitamin use

☺ Alcohol – your alcohol consumption is within the recommended limits, but we have found a variation in an alcohol metabolism gene

😐 Fruit & vegetables – your consumption of fruit and vegetables is low

☹ Wholegrains – you need to increase the amount of whole grains in your diet

😐 Sugar intake – your consumption of sugar is high

☹ Saturated fats – you include too many saturated fats in your diet

☹ Body weight – your BMI places you in the overweight range

☹ Physical activity – increase your level of physical activity

Table 7

Claims:

1. A computer-assisted method of providing a personalized lifestyle advice report for a human subject comprising:

(i) providing an identifier to a sample of DNA obtained from the human subject,

(ii) linking said identifier to personal details of the subject held in a secure location in a data processing means,

(iii) identifying individual alleles at one or more genetic loci in said sample to generate a first dataset of said human subject, wherein at least one allele of each genetic locus is known to be associated with factors which affect wellbeing or lifestyles in a positive or negative way,

(iv) linking said first dataset to said identifier,

(v) linking said first dataset and said personal details in said secure location by means of said identifier,

(vi) providing a second dataset on a data processing means, said second dataset comprising information correlating the presence of individual alleles at said genetic loci with a lifestyle risk factor;

(vii) providing a third dataset on a data processing means, said second dataset comprising information matching each said risk factor with at least one lifestyle recommendation;

(viii) determining the risk factors associated with said alleles of said human subject in said first dataset using said second dataset;

(ix) determining at least one appropriate lifestyle recommendation based on each identified risk factor from step (viii) using said third dataset,

(x) generating a personalized lifestyle advice plan based on said lifestyle recommendations and said personal details, and;

(xi) producing a report document containing said lifestyle advice plan.

2. A method according to claim 1 comprising inserting and sealing said document in an enclosure in an automated process.

3. A method according to claim 2 wherein said enclosure is labelled with one or more of postal details of said human subject and said identifier.

4. A method according to claim 4 comprising sending said report document to said human subject.

5. A method according to any one of claims 2 to 4 wherein the enclosure comprises a non-resealable opening mechanism.

6. A method according to claim 4 wherein the enclosure comprises a tamper-proof seal.

7. A method according to any one of claims 1 to 5 wherein said identifier is a barcode.

8. The method according to any one of claims 1 to 7 wherein the personalised lifestyle advice plan includes recommended minimum and/or maximum amounts of food subtypes.

9. The method according to any one of claims 1 to 8 wherein said first dataset comprises information relating

to two or more alleles of one or more genetic loci of genes selected from each member of the group consisting of:

(a) genes that encode enzymes responsible for detoxification of xenobiotics in Phase I metabolism;

(b) genes that encode enzymes responsible for conjugation reactions in Phase II metabolism;

(c) genes that encode enzymes that help cells to combat oxidative stress;

(d) genes associated with micronutrient deficiency;

(e) genes that encode enzymes responsible for metabolism of alcohol.

(f) genes that encode enzymes involved in lipid and/or cholesterol metabolism;

(g) genes that encode enzymes involved in clotting;

(h) genes that encode enzymes related to susceptibility to metal toxicity;

(i) genes which encode proteins required for normal cellular metabolism and growth;

(j) genes that encode proteins involved with inflammation processes

(k) genes involved in calcium metabolism and bone growth and maintenance.

10) The method according to claim 9 wherein said first dataset comprises information relating to two or more alleles of the genetic loci of genes encoding the enzymes: 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), cystathione beta synthase (CBS), vitamin D receptor (VDR), collagen type I alpha (COL1A1), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), angiotensin converting enzyme (ACE), peroxisome proliferators activated receptor (PPAR-gamma 2), manganese superoxide

dismutase (SOD2), extracellular superoxide dismutase (SOD3), glutathione S-transferase M1 (GSTM1), glutathione S-transferase thetal (GSTT1), glutathione S-transferase pi (GSTP1), apolipoprotein C-III (APOC3), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), endothelial nitric oxide synthase (eNOS), factor 5 (F5) and apolipoprotein E (ApoE4).

11. The method according to any one of claims 1-10 wherein said presence of said individual alleles is determined by hybridisation with allele-specific oligonucleotides.

12. A computer-assisted method of providing a personalized lifestyle advice report for a human subject comprising:
(i) providing a first dataset on a data processing means, said first dataset comprising information correlating the presence of individual alleles at genetic loci with a lifestyle risk factor, said genetic loci consisting of two or more of;

(a) genes that encode enzymes responsible for detoxification of xenobiotics in Phase I metabolism;

(b) genes that encode enzymes responsible for conjugation reactions in Phase II metabolism;

(c) genes that encode enzymes that help cells to combat oxidative stress;

(d) genes associated with micronutrient deficiency; and

(e) genes that encode enzymes responsible for metabolism of alcohol.

(f) genes that encode enzymes involved in lipid and/or cholesterol metabolism;

(g) genes that encode enzymes involved in clotting;

(h) genes that encode enzymes related to susceptibility to metal toxicity;

(i) genes which encode proteins required for normal cellular metabolism and growth;

(j) genes that encode proteins involved with inflammation processes

(k) genes involved in calcium metabolism and bone growth and maintenance.

(ii) providing a second dataset on a data processing means, said second dataset comprising information matching each said risk factor with at least one lifestyle recommendation;

(iii) inputting a third dataset identifying alleles at one or more of the genetic loci of said first dataset of said human subject;

(iv) determining the risk factors associated with said alleles of said human subject using said first dataset;

(v) determining at least one appropriate lifestyle recommendation based on each identified risk factor from step (iv) using said second dataset; and;

(vi) generating a personalized lifestyle advice plan based on said lifestyle recommendations.

13) The method according to claim 12 wherein said genetic loci consist of: 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), cystathione beta synthase (CBS), vitamin D receptor (VDR), collagen type I alpha (COL1A1), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), angiotensin converting enzyme (ACE), peroxisome proliferators activated receptor (PPAR-gamma 2), manganese superoxide dismutase (SOD2), extracellular superoxide dismutase (SOD3), glutathione S-transferase M1 (GSTM1),

glutathione S-transferase theta1 (GSTT1), glutathione S-transferase pi (GSTP1), apolipoprotein C-III (APOC3), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), endothelial nitric oxide synthase (eNOS), factor 5 (F5) and apolipoprotein E (ApoE4).

14. The method according to any one of claims 12 or 13 including the step of determining the presence of individual alleles at one or more genetic loci of the DNA in a DNA sample of said human subject, and constructing the dataset used in step (iii) using results of said determination.

15. The method according to claim 14 wherein said presence of said individual alleles is determined by hybridisation with allele-specific oligonucleotides.

16 The method of any one of claims 12 to 15 comprising
(vii) producing a report document containing said lifestyle advice plan; and,
(viii) applying a tamper-proof seal to said report document.

17. A microarray comprising a plurality of oligonucleotides, said plurality comprising oligonucleotides specific to an individual allele of each of the genes selected from the group consisting of:

(a) genes that encode enzymes responsible for detoxification of xenobiotics in Phase I metabolism;

(b) genes that encode enzymes responsible for conjugation reactions in Phase II metabolism;

(c) genes that encode enzymes that help cells to combat oxidative stress;

(d) genes associated with micronutrient deficiency; and

(e) genes that encode enzymes responsible for metabolism of alcohol.

(f) genes that encode enzymes involved in lipid and/or cholesterol metabolism;

(g) genes that encode enzymes involved in clotting;

(h) genes that encode enzymes related to susceptibility to metal toxicity;

(i) genes which encode proteins required for normal cellular metabolism and growth;

(l) genes that encode proteins involved with inflammation processes; and

(k) genes involved in calcium metabolism and bone growth and maintenance.

18. A microarray according to claim 17 wherein said plurality comprises oligonucleotides specific to an individual allele of each of the genes selected from the group consisting of:
5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), cystathione beta synthase (CBS), vitamin D receptor (VDR), collagen type I alpha (COL1A1), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), angiotensin converting enzyme (ACE), peroxisome proliferators activated receptor (PPAR-gamma 2), manganese superoxide dismutase (SOD2), extracellular superoxide dismutase (SOD3), glutathione S-transferase M1 (GSTM1), glutathione S-transferase theta1 (GSTT1), glutathione S-transferase pi (GSTP1), apolipoprotein C-III (APOC3), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL),

endothelial nitric oxide synthase (eNOS), factor 5 (F5) and apolipoprotein E (ApoE4).

19. A set of at least 10 primer pairs comprising a plurality of oligonucleotides, said plurality consisting of primer pairs specific for individual alleles of each of the following genes;

(a) genes that encode enzymes responsible for detoxification of xenobiotics in Phase I metabolism;

(b) genes that encode enzymes responsible for conjugation reactions in Phase II metabolism;

(c) genes that encode enzymes that help cells to combat oxidative stress;

(d) genes associated with micronutrient deficiency; and

(e) genes that encode enzymes responsible for metabolism of alcohol.

(f) genes that encode enzymes involved in lipid and/or cholesterol metabolism;

(g) genes that encode enzymes involved in clotting;

(h) genes that encode enzymes related to susceptibility to metal toxicity;

(i) genes which encode proteins required for normal cellular metabolism and growth;

(j) genes that encode proteins involved with inflammation processes; and

(k) genes involved in calcium metabolism and bone growth and maintenance.

20. A set according to claim 19 wherein said plurality consists of primer pairs specific for individual alleles of each of the following genes; 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine

synthase reductase (MTRR), cystathione beta synthase (CBS), vitamin D receptor (VDR), collagen type I alpha (COL1A1), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), angiotensin converting enzyme (ACE), peroxisome proliferators activated receptor (PPAR-gamma 2), manganese superoxide dismutase (SOD2), extracellular superoxide dismutase (SOD3), glutathione S-transferase M1 (GSTM1), glutathione S-transferase theta1 (GSTT1), glutathione S-transferase pi (GSTP1), apolipoprotein C-III (APOC3), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), endothelial nitric oxide synthase (eNOS), factor 5 (F5) and apolipoprotein E (ApoE4).

21. A set according to claim 19 or 20 wherein the set comprises at least one primer pair of SEQ ID NO:n and SEQ ID NO:(n+1), where n is 1 to 53.

22. A method of profiling an individual's risk factors to dietary and environmental factors which method comprises bringing a sample of the individual's DNA into contact with an array according to any one of claims. 17 to 18 or set of primer pairs according to any one of claims 19 to 21, determining the presence or absence of alleles of genes detectable by said array or pairs associated with risk factors present in the individual, and performing the method of any one of claims 1 to 16.